

nature

23 June 1977

Dismantling the firebreak

RESEARCH and development of tactical nuclear weapons has been conducted for many years now with relatively little public debate against a background of even less public information. So it would be unfortunate if the recent revelation of budgetary proposals for 'enhanced radiation' devices to be built by the US Energy Research and Development Administration were to be seen only as a step, and maybe not a very big one at that, towards a new type of weapon. For the whole question of tactical nuclear weapons, regardless of their mixture of blast and radiation content, deserves a much wider airing.

Tactical nuclear weapons have yields of the order of tons rather than the kilotons or megatons of strategic devices. They can be fired from tanks as shells; and as miniaturisation proceeds they are more easily adapted to mortars or even suitcases. Enhanced-radiation/reduced-blast tactical devices would carry the added attraction for the field commander that the area bombarded would not be so devastated and full of radioactive debris as to be inaccessible subsequently to his own troops. So an enhanced radiation device would be ideal for land battles where territorial advantage counted.

Not so ideal, however, is the way recent developments in nuclear weapons have been closing the gap between

conventional explosives and strategic nuclear weapons. Until the late 1960s there was a very clear firebreak between the limited damage that chemistry could inflict and the devastation that physics could wreak. Soldiers could be entrusted with conventional weapons, but only presidents could launch nuclear devices. Then to bridge this gap came tactical weapons that were scaled down strategic weapons.

Now the talk is of devices that cause even less damage but kill more, and they are beginning to look distinctly like biological weapons. Advocates of the new devices still see firebreaks to prevent a limited confrontation from escalating into an all-out nuclear war, but this is a very optimistic point of view and assumes that belligerents will recognise each other's devices for what they are, have no wish to respond more aggressively and have the right weapons available to permit response in kind. It's a tenuous line of argument to say the least, and many will find it totally unconvincing.

In the past 25 years an immense amount of thought has been devoted to the possibilities of restricting strategic armaments. The need now is for some equally intensive studies to be made of ways of averting the escalation which new weapons technology is making progressively more likely. □

Dissecting the crisis

RECENTLY the European Commission, the EEC's administrative-cum-executive arm, sponsored a colloquium in Brussels entitled 'Crisis of science in European societies?' Everyone who attended seemed to take it for granted that the answer to the question is 'yes'. But though that may be right, the crisis borne of the modern problems of the bomb, the environment, energy, resources and changing social attitudes has been around for some years now. And crises, like lost tempers, should be over quickly if they are not outward signs of something more serious.

The Brussels symposium could hardly have been said to have come to grips with this deeper problem, beyond revealing that there are different opinions and judgments about what should be done. For some this may be suf-

ficient—a symbolic meeting of cultures, an agreeable renewal of old friendships, may be all they sought from such a meeting. They might even see it as pretentious to claim that gatherings like these could go further, though just such hopes were being entertained.

But there is meanwhile a real and deepening problem for many scientists. It involves falling funds, less research and fewer researchers. And it highlights what is perhaps the most serious problem to be faced: that scientists, unlike most other discernible groups, do not seem able to organise themselves to talk plainly to governments. Nor for that matter does the decision-making system encourage such organisation. Amiable gatherings in Brussels are a poor substitute for hard talking nearer home. □



Too late not to proliferate?

David Fishlock examines the background to the burgeoning international trade in nuclear technology

DR Glenn Seaborg, the chemist who with E. M. McMillan first isolated plutonium, delivered a paper on "the plutonium economy of the future" in Santa Fe in 1970. It was an important paper; Seaborg, as chairman of the US Atomic Energy Commission, was forecasting that by 1990 one-quarter of the energy used by the USA could come from plutonium. By then, he said, the nation would have accumulated a plutonium stockpile from its light water reactors worth (at 1970 prices) about \$6,000 million—twice as much as the US investor-owned utilities had spent on fuel in 1969.

The paper attracted little attention. The campaign against nuclear energy in the US, although well under way, was almost wholly engaged in attacking the intrinsic safety of nuclear reactors. Proliferation of nuclear materials, although a subject of deep concern to a great many governments, was not a public issue, much less a target of anti-nuclear campaigners. Neither were nuclear weapons or nuclear materials then seen as serious targets for international terrorists.

By the mid-1970s, however, the anti-nuclear campaigners badly needed a new target. Although they had succeeded in slowing the pace of licensing and construction of reactors, they had failed completely to make out a case against them as a potential hazard. Not a single nuclear plant had been shut down because it was emitting, or was considered liable to emit, dangerous amounts of radiation.

Plutonium, and plans to refine and recycle it, and to use it to fuel more advanced types of reactor, offered a tempting new target. The very name of the element, its "fiendishly toxic properties" as one US physicist had once described them, and its immense potential as an explosive offered clear advantages in arousing public fears. Overnight the 'plutonium economy' became a catchphrase depicting a world unfit for human beings.

Three events

Three events contributed importantly to the widespread fears now aroused about the plutonium economy and its implications. The first, in May 1974, was the explosion by India of a plutonium device it euphemistically referred to as a "peaceful nuclear explosive". This was a profound shock

for those nations which for nearly 20 years had been striving for international controls on the use of nuclear technologies. In public India had declared repeatedly that it would never make a nuclear weapon. So, in spite of the fact that India had been opposed from the outset to any idea of international control and safeguards through the UN's International Atomic Energy Agency (IAEA), such nations as the US and Canada had provided India with nuclear technology and know-how, sometimes on very generous terms. Under the Colombo Aid programme Canada provided an NRX research reactor free from international safeguards. The Indians used this reactor to transmute natural uranium into plutonium.

The second event occurred about a year later, in the summer of 1975, when after some months of publicity the West German Government signed a contract with Brazil to provide that country with virtually a ready-made nuclear industry, including the technology to separate plutonium and to enrich uranium. It was the biggest nuclear export contract any country had ever won. But the US Government had already turned down Brazil's requests for enrichment and reprocessing, not least because Brazil refused to sign the 1969 Non-Proliferation Treaty (NPT).

The furore over the German-Brazilian contract also highlighted a number of other nuclear contracts under negotiation, notably contracts under which France planned to provide Pakistan and South Korea with reprocessing plants. Together with the Indian explosions, these negotiations prompted the US Government to bring together for the first time the seven leading nuclear supplier nations at a secret meeting in London to try to find more effective controls for the transfer of 'sensitive nuclear technologies'. These were then defined as the technologies of reprocessing and enrichment, both of which could be adapted to yield fissile materials, and the production of heavy water.

The third event of importance in establishing the plutonium economy as a public issue was the publication in September 1976 of the Sixth Report of the Royal Commission on Environmental Pollution, under the chairmanship of Sir Brian Flowers. In spite of the fact that Flowers had never once raised the matter as a part-time mem-

ber of the UK Atomic Energy Authority, this report expressed deep concern with the idea of any plutonium falling into the hands of terrorists. If the stability of our society continued to deteriorate, it suggested, proliferation of the sources and movements of plutonium could begin to pose a serious hazard.

Meanwhile the US Government had been abruptly awakened to the fact that it was fast losing control of technologies it had sought to control—for example, through its patronage of the IAEA and its domination of the enrichment and reactor markets—since the end of the Second World War. It searched desperately for fresh safeguards against proliferation. Dr Henry Kissinger, as Secretary of State, enthusiastically endorsed an IAEA idea for regional fuel cycle centres operating under international management and safeguards to serve such areas as Latin America and the Middle East with 'sensitive technologies'. A major study by the IAEA soon disclosed that while the regional concept was politically impractical—it was difficult to conceive of a project or a site that would serve Brazil, Argentina and Chile, for instance, or Egypt, Israel and Iran—the multinational reprocessing centre was a serious proposition. Where the centre was located was of small importance since transport costs varied little with distance. But economies of scale made the economic advantages of large plants attractive to any nation with purely commercial aims. The IAEA concluded that ideally a multinational reprocessing centre would be set up by five or seven nations, one or more of which would be able to provide the technology and organise the financing of plants expected to cost of the order of \$1,000 million. Such a centre would justify full-time surveillance by IAEA inspectors to ensure that no illegal diversion of plutonium took place.

Interest waned

Promising as the outcome of this study proved to be, the interest of the US Government had waned long before its formal presentation at the nuclear conference in Salzburg next month. At best it could be a medium-term solution, for such centres would need at least a decade to organise and bring into service. Even the technologically simpler problem of organising international plutonium storage might take a decade to bring about. The US was urgently searching for tighter controls against proliferation that could be implemented here and now.

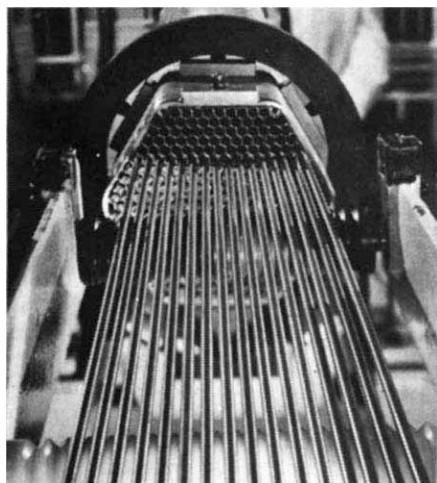
Its response came finally in a statement on 7 April in which President Carter formally declared a moratorium on one of the sensitive technologies,

reprocessing. In future the US would store the highly radioactive fuel from its light water reactors. The president took care at this time to stress that this was domestic US policy for controlling proliferation, and that it recognised that other nations with no indigenous uranium might not be so willing to forego the uranium and plutonium locked up in spent fuel.

During the month that followed two further aspects of US anti-proliferation policy emerged. One was that in order to try to persuade other nations to follow the US policy, America was going to expand her uranium enrichment capacity while still keeping this activity firmly under government control. The implication was that it was prepared to supply enriched uranium from its own reserves, enriched to the low level required in existing thermal reactors, and moreover would be prepared to store unprocessed spent fuel for customers.

What also emerged, in a statement from Dr Joseph Nye, a senior State Department official concerned with anti-proliferation, was that the US would bring pressure to bear on foreign governments to follow its policy by refusing permission to reprocess, either at home or elsewhere, fuel which had previously been enriched in the US. In practice, this meant most of the enriched fuel extant in the West.

This ban could be implemented under the bilateral agreements covering enrichment of the original fuel. Where customers, said Dr Nye, were in special difficulties from lack of storage capacity, and plants might have to be shut down, the US Government would be prepared to consider special dispensation. But licences to re-export the fuel for reprocessing would be granted only in cases of "clear need". There could be no question of a general dispensation of the kind necessary to support the 10-year contracts under negotiation between the Japanese electricity industry and Britain and France.



Plutonium fuel pins

These statements evoked widespread and sometimes vehement criticism from countries which would be immediately affected, notably those having no indigenous uranium but busy laying foundations for plutonium-fuelled fast breeder reactors to limit their dependence on uranium imports. One specific criticism levelled against the US was that for reasons of domestic expediency it was prepared to renounce one sensitive technology while expanding another, enrichment; and using, moreover, a technology—the gas centrifuge—which only a few years before it had argued would pose a serious threat to the control of proliferation. More generally it was asked what guarantees a nation could have for placing its faith in the US, whose own motives and intentions regarding fast reactors are uncertain.

Developing nations, whatever their nuclear aspirations, indignantly attacked what they saw as a rich nation's attempt to debar them from a valuable technology. The USSR, which has its own uranium reserves and also reprocesses spent fuel for its satellite nations, keeping the plutonium in Russia, has indicated that it will have nothing to do with President Carter's proposals. The policy could only hamper its own well-developed plans for fast reactors.

As with Russia, Britain has longstanding plans for safeguarding the future of its energy supplies once North Sea reserves run out by burning plutonium in fast reactors. Recycled in this way, Britain's existing stockpile of uranium has an energy content exceeding that of proven coal reserves. Britain also has plans to use its knowledge of the difficult technology of reprocessing to develop an international service, under IAEA safeguards, for nations which would prefer not to embark on this technology, at least at this stage. These plans could be severely hampered by US refusal to grant re-export licences for reprocessing to nations whose fuel has been enriched in the US.

Dr David Owen, the UK Foreign Secretary, in the fullest public response yet by any nation, on 10 May pointed out bluntly some of the weaknesses, as Britain sees it, of President Carter's anti-proliferation policy. He emphasised that Britain remained firmly committed to the principles of non-proliferation. But an effective anti-proliferation policy "must go hand-in-hand with a viable energy strategy", he said. He pointed to routes to nuclear explosives which the US policy would apparently leave uncontrolled. And he further implied that President Carter, far from improving relations, has seriously worsened them in this highly sensitive area of international affairs.

Continued proliferation?

A more realistic approach to the problem might be to assume that nuclear weapons will continue to proliferate. Although their attractions to terrorists have probably been greatly exaggerated, the attraction to governments remains undisputed. And governments would find it far easier to assemble the resources required to make credible nuclear explosives. In 1945 only one nation possessed these explosives. Through the McMahon Act the US debarred Britain, a partner in the Manhattan Project, from further participation in the technology. The UK Government, convinced that it needed the technology for broad reasons of national security, promptly embarked on its own nuclear programme. The USSR and France took similar decisions; and later China and India. An impressive 104 nations have signed the NPT, eschewing nuclear explosives; but another 30 have not, among them some with well-developed nuclear plans.

No moratorium on all, still less on parts, of the nuclear technologies by states already possessing nuclear weapons is credible as a way of stopping one of those 30 nations if it is determined, as Britain once was, to acquire the Bomb. It could even encourage it in such a quest. Neither need these nations try to buy large nuclear power stations costing around \$1,000 million as the source of plutonium. As a study specially commissioned by the US Congressional Research Service concluded last year, a small nation with ambitions for nuclear weapons could satisfy them for an investment as small as \$13 million—\$26 million, by building a small gas-cooled, graphite-moderated type of reactor, capable of making about 10 kilogrammes of plutonium a year. The design data for such a 'plutonium furnace' is already freely available in the open literature, and all the materials and components with the single exception of uranium—which is widely distributed across the Earth's surface—can be bought on the open market.

The UK Government estimates that about a dozen nations already have the basic knowledge and access to fissile materials. By 1987 another 20 could have reached this position. In short, it is already too late to prevent other nations from joining the nuclear club if they are determined to do so. A far more persuasive deterrent would be to create an international climate sufficiently hostile to nuclear proliferation to persuade any would-be aspirants that they would lose much more than they could possibly gain by possession of nuclear explosives. So far this has manifestly not been the case. □

USA

Feeling the draft

Legislation to control experiments with recombinant DNA is progressing slowly through Congress. Colin Norman reports

THREE months ago, when Congress first began to consider legislation to regulate recombinant DNA experiments in the United States, many scientists were hoping that a straightforward bill, establishing a uniform set of national controls, would swiftly be put together and passed without too much wrangling. Congress has not obliged, however. When the bill finally emerges, it will be a lengthy and complex document, establishing an elaborate mechanism for setting new regulations, ensuring that they are followed, and providing punishment for those caught flouting the rules. Moreover, a good deal of political manoeuvring is still in store before the bill emerges in final form from the Congressional mill.

At least the legislative machinery is beginning to pick up speed, however. Last week, key committees in both the House and the Senate passed their own versions of legislation, paving the way for consideration of the measures by the full House and Senate in the next few weeks. The House subcommittee on Health, headed by Representative Paul G. Rogers, approved a bill running to 46 pages; it will now be considered by the full Commerce Committee and possibly by the Committee on Science and Technology, before being sent to the House floor. It is unlikely that the draft approved by the health subcommittee will be substantially modified at any of those stages, however. In the Senate, the Committee on Human Resources last week approved a 56-page bill drafted by staff members of the Senate Health subcommittee, headed by Senator Edward Kennedy. It will probably be sent to the full Senate next month.

The two versions differ significantly in their approach to regulating recombinant DNA research, and thus a Senate-House conference committee will eventually be given the task of putting together a compromise bill.

Though there are substantial differences in the two bills, there are a number of important points of similarity. Both would require new safety regulations to be established, governing all recombinant DNA experiments in the United States. They also both require that facilities in which recombinant DNA experiments are con-

ducted must be licenced, that violators be subject to heavy fines, that extensive records be kept, and that federal inspectors have a right to investigate facilities and records. They also call for a study of long-term implications of genetic engineering, and they both come close to specifying that the federal regulations should take precedence over controls imposed by state or local governments. The big difference is that the Senate version would establish a Presidentially-appointed regulatory commission to draft and enforce the regulations, while the House version would leave much of the day-to-day enforcement to local biohazards committees.

The House bill, though largely written by committee staff members, was put together during three lengthy committee meetings at which about half-a-dozen Congressmen took an active interest. The chief provisions:

- The Secretary of Health, Education and Welfare (HEW) would be required to draft new regulations governing recombinant DNA research. They would prescribe physical and biological containment conditions for different categories of experiments, set out training requirements for laboratory workers and researchers, specify systems to monitor for the escape of potentially hazardous organisms, require medical surveillance of laboratory personnel, and describe reports and records which must be maintained in research facilities. The regulations would have to be published within one year after the bill is passed, and they would come into effect six months later. In the meantime, the NIH guidelines would be made applicable to all recombinant DNA experiments, no matter whether they are conducted in public or private institutions.

- A part-time advisory committee, consisting of 17 members appointed by the Secretary of HEW, would be established to assist in drafting and implementing the regulations. A majority of the members would be people who are not engaged in recombinant DNA research, and the committee would include a laboratory worker and a representative from industry.

The guts of the bill is a provision establishing a mechanism for licencing facilities housing recombinant DNA experiments. An application for a licence would have to contain a thorough description of proposed experiments, including experimental protocols, assurances that the regulations would be followed, and an analysis of the potential risks to public health or

the environment arising from each proposed experiment. The application and granting procedure would vary according to the type of experiment proposed, but in every case, local biohazards committees would play a strong rôle.

These would be committees consisting of between 9 and 15 members, including at least one laboratory worker and one local public health official. A majority of the committee members would be scientists. The biohazards committee would review all licence applications for facilities under its purview, and it would have the power to grant licences for experiments requiring low containment levels—P1 and P2 under the NIH guidelines. For research requiring P3-level containment, the licence application would have to be reviewed first by a biohazards committee, and subsequently by the Secretary of HEW and his advisory committee. The Secretary would be able to override a negative recommendation by his advisory committee, but in practice such a decision would be unlikely. A similar procedure would be required for applications involving P4-level work, but in such cases the Secretary would be able to grant a licence only if his advisory committee recommended approval.

It should be noted that, by specifying different arrangements for experiments requiring P1, P2, P3 and P4 levels of physical containment, the legislation presumes that such levels will form the basis of the final regulations.

The House bill would leave most of the inspection of facilities and monitoring to the biohazards committees, though federal inspectors would also be empowered to check to ensure that the regulations are followed. Violations could result in revoking a facility's licence, and in addition researchers could be subject to fines amounting to \$5,000 per day for each offence. Particularly serious and wilful violations could result in a \$50,000 fine and up to a year in prison. The bill also provides legal protection for workers who blow the whistle on their employers by bringing incidents of flouting the rules to public attention.

The bill approved by the Senate committee would establish a very different regulatory framework. It would set up an 11-member National Recombinant DNA Safety Regulation Commission to draft regulations, issue licences for facilities and monitor compliance with the regulations. The chairman would be a full-time official, while the other commissioners would be part time; all would be appointed by the President. A majority of the commission members would be non-

biologists.

The commission would be required to issue new regulations within 120 days after the bill is approved; they must be at least as strict as the NIH guidelines. The Senate bill also requires that all facilities housing recombinant DNA experiments must be licenced, and licence applications must specify the proposed experiments, the names of investigators and their qualifications. The Commission would be responsible for granting all licences, but initial review and approval of recombinant DNA projects would be undertaken by local biohazards committees. At least one third of the members of the local committee must be non-biologists, and at least another third would be employees of the facility applying for the licence.

The Senate bill also specifies that federal inspectors would have the right to enter facilities to inspect records and equipment. It also requires extensive record-keeping by researchers. As in the House bill, the Senate version specifies that violations could result in denial of a licence, and violators could be subject to a fine of \$10,000 per day.

Though the two bills would probably not lead to the adoption of radically different regulations, they would lead to different forms of bureaucratic control. In particular, a number of scientists have expressed concern that if a large commission, backed by a full-time staff, is established to review and approve each licence application, there could be considerable bureaucratic delay. One scientist also suggested last week that a Presidential commission to oversee recombinant DNA research would amount to 'overkill'.

One of the most controversial points in the legislation has been the question of whether federal regulations should override controls imposed by state or local governments. In fact, that was the only point which was even discussed during the Senate committee's consideration of the bill last week. The House and Senate versions are not greatly different in their requirements. The Senate bill would allow local governments to set stricter controls only if the extra safety precautions are "material and relevant" to deal with "health and environmental concerns or comparable compelling local conditions", and if they are not "arbitrary or capricious". The House bill would allow stricter local controls only if the Secretary of HEW determines that they are "necessary to protect public health or the environment".

The bills are not likely to reach the floor of either the House or the Senate much before the end of July, and it will then take several weeks for a conference committee to draft a compromise between the two versions. □

● An intensive lobbying campaign by space scientists has won a reprieve for two high priority projects, the Large Space Telescope and the Jupiter Orbiter Probe. A key Senate appropriations subcommittee last week voted funds for both projects, in spite of opposition from Senator William Proxmire, the subcommittee chairman. The vote will almost certainly ensure that the telescope will go ahead as planned, but prospects for the Jupiter project are a little less certain.

The Large Space Telescope seems assured of Congressional support because it has already been approved by the House of Representatives. Last week, the House passed a budget bill for NASA which contains funds to begin building the instrument in the 1978 fiscal year. Thus, if the full Senate follows the recommendation of Proxmire's subcommittee, which is likely, both the House and Senate versions of the NASA budget bill would contain funds for the telescope, thereby virtually guaranteeing final Congressional approval for the project.

The Jupiter Orbiter Probe is still in some trouble, however, because no funds for the project were included in the House bill. The House Appropriations Committee had specifically deleted the money from NASA's budget request, arguing that approval of two major space science projects in one year cannot be justified. The space telescope has higher priority, the committee said, and therefore the Jupiter project should be dropped. The full House followed the committee's recommendation.

It thus seems likely that the Senate version of the NASA budget bill will contain funds for the Jupiter project, the House version will not, and the matter will have to be decided by a House-Senate conference committee. NASA is hoping that it can persuade the conference committee to approve the project.

It is likely that the conference committee will in fact provide some funds for the project, but not as much as the \$20.7 million that NASA requested for 1978. In that case, NASA would at least be able to begin building the spacecraft and it would be able to avoid laying off scientists and engineers at the Jet Propulsion Laboratory, which will manage the project. The spacecraft is scheduled to be launched late in 1981 or early in 1982.

● President Carter's plan to slow down the development of a commercial liquid metal fast breeder reactor is,

like other key elements of his energy policy, being severely mauled on Capitol Hill. Carter had proposed that a demonstration breeder reactor, scheduled to be built on the Clinch River in Tennessee, should be deferred indefinitely, and he recommended that virtually all the funds for the project should be deleted from the budget for the Energy Research and Development Administration. Last week, however, the House Committee on Science and Technology voted by 19 to 11 to approve the Clinch River plant and it restored \$150 million to ERDA's budget to begin constructing the facility next year.

The vote far from guarantees that the Clinch River plant will be saved from the axe, but it does at least ensure that there will be a full-scale debate on the matter when the ERDA bill reaches the House Floor. Senator Howard Baker, who represents Tennessee, reportedly said last week that the vote has made him "optimistic for the first time" that the plant will be reprieved.

Carter's recommendation that the Clinch River plant should be cancelled was a key part of his plan to prevent the use of plutonium as a reactor fuel. He also proposed that the United States should defer indefinitely the reprocessing of spent reactor fuels to recover plutonium for recycle in light water reactors. At the same time, the Administration is trying to dissuade other countries, particularly those which do not possess nuclear weapons, from entering the so-called plutonium economy.

The House Committee on Science and Technology was persuaded, however, that other countries would not defer their plans to recycle plutonium and build breeder reactors. The committee therefore argued that the United States should not allow itself to be deprived of a technology which other industrialised countries are vigorously pursuing.

Similar statements have also been made by Senator Frank Church, who heads a key energy research subcommittee in the Senate, and by Senator Henry Jackson, who chairs the Senate Committee on Energy and Natural Resources. Their support for the breeder programme is almost certain to force a full debate on the Clinch River plant in the Senate.

Thus a protracted debate on Carter's plutonium policy seems assured in both the House and the Senate. At this stage, nobody is willing to predict the outcome.

Colin Norman

EEC

Council flat

The EEC Council of Energy Ministers and Council of Environment Ministers both met in Luxembourg last week. Patricia Kelly and Chris Sherwell report

THE two councils held last week shared a common vein. There were no concrete decisions; and a mass of texts was referred back to national experts for further discussion. Thus it was hardly surprising that one of the most useful discussions demanded no decisions in the first place. That was the debate in the energy council on nuclear energy.

The debate had a status analogous to the open discussion at the March council on energy conservation. The Energy and Research Commissioner, Dr Guido Brunner, worried about negotiations with the US and Canada, wanted the session behind closed doors, but no one agreed. Discussion centred on Community problems relating to uranium supplies, reprocessing and waste management.

More specifically, the idea of Community responsibility for safety aspects of the fuel cycle was discussed. Unsurprisingly, some concern was expressed at the idea of handing over responsibility on a matter where political control could prove so important. The question of Community participation in the high level study set up by the recent London summit was also raised, but not pursued.

On nuclear power forecasts, Britain, together with France and Germany, found the Commission's 1985 projections optimistic. Britain also criticised a suggestion, in a Community paper prepared for the meeting, for a high temperature reactor project—memories of Dragon are still fresh. There was no discussion of the vexed problem of nuclear accounting, over which a controversy, involving past uranium shipments, had blown up since the last council. The matters of nuclear costs, of public opinion and of the fast breeder could only be mentioned.

In restricted session the UK Government refused to accept what it interpreted as an enforced share-out of its North Sea oil with the rest of the Community in the event of a new world energy crisis. It was agreed that in the case of an emergency, EEC headquarters would order a 10% cut in oil consumption for two months. The Commission wanted the percentage of the cut for those countries that cannot easily find substitutes for oil to be calculated in terms of that country's oil

consumption only; in countries where substitutes are more readily found, this percentage would depend on total energy consumption including oil. Italy's Carlo Donat Cattin was anxious that the Italian dependence on oil should be safeguarded in the event of a crisis. But Britain's junior minister, Dr Dickson Mabon, said that as the Community's only major energy producer, the UK was not prepared to cut its consumption more than other countries at the dictation of the Community.

It was clear that while the UK Government would be ready to help those countries most affected by an energy crisis, it could not accept a decision taken by a qualified majority vote in the council. Britain was the only country that did not want the council to depart from unanimous voting. Ministers instead agreed that if a structural problem was affecting any member state, should the Commission order a cut, the council would undertake to meet within ten days of specific proposals from the Commission.

On the matter of the minimum safeguard price for oil there was apparently little effective discussion. But the Dutch minister, Rudi Lubbers, raised the question of long-standing Dutch proposals on energy prices generally. He argued that EEC energy prices should be brought up to oil parity to encourage conservation. But Dr Mabon commented that it was too early for common pricing. The council also disagreed about Commission proposals on how to tackle overcapacity in the Community's oil refining industry. The talks foundered on the consultation system where governments would have to seek the Community's approval for new refineries to be built. On top of this, there was no discussion at the council of proposals to regulate financial support for projects exploiting alternative sources of energy.

Three coal texts were among proposals sent back for further discussion. These were the monitoring of imports from third countries, the creation of an EEC financial aid scheme to encourage the burning of coal in new power stations and a system of EEC financial support for coal stocks.

The UK Energy Secretary, Anthony Wedgwood Benn, in the chair for the last time before the new Belgian Minister for Economic Affairs, Willy Claes, takes over the council presidency, said that it had been a solid six months of work during the UK presidency. At Mr Benn's initiative, the council has agreed to hold a general debate at each energy council, on EEC

energy and on world energy.

As for the Environment Council, this seemed doomed from the start. One observer commented that 'red mud', a by-product of the titanium dioxide industry, not only polluted the marine environment but also seemed capable of polluting the atmosphere—notably between ministers. The Italian delegation made it clear that if the council failed to agree with Commission proposals to control waste from the TiO_2 and paper pulp industries—issues on which agreement has long been sought—then it would not agree to a single item on the agenda. The Italians stuck to their guns.

The Nine were split on the old question of uniform emission standards versus quality objectives as a means of control. The British argument, backed up by Ireland and Denmark, is that their industries discharge into tidal waters able to cope with large quantities of pollutants, making the Commission proposals environmentally unnecessary as well as costly to fulfil. France and Italy would like to see all Community countries impose the same controls to avoid putting industries at a competitive disadvantage. Britain maintains that what it gains on environmental cost advantages is lost on transport against more centrally placed Community countries, and was quick to point out that the dual approach to pollution control was accepted by the Nine in 1975 in a directive on the discharge of dangerous substances into the aquatic environment.

Thanks partly to the work of a working group set up during the council, some way of proceeding further may be possible, based on a distinction between reducing pollution caused by waste from an industry and reducing the quantity of the waste.

The council continued in spite of the Italian reserve. Ministers approved a decision establishing a common procedure for the exchange of information on the quality of surface fresh water in the Community. It will be formally adopted when the Italian reserve is lifted. They failed to finish discussion on EEC accession to the Helsinki Convention on protection of the Baltic. Authorisation to open negotiations is expected. Differences between the Dutch and countries through which the Rhine flows before reaching Holland meant no agreement was possible on proposals on the quality of drinking water. The proposals go back to Coreper, the committee of EEC ambassadors, for further discussion. So do proposals on toxic and dangerous waste on which the council did reach a consensus on the main aspects; a directive is expected soon. □

JET

Stalling point

RIDING on the back of a busy day in Brussels last week when the Councils of Energy and Environment Ministers both met (see opposite), the controversial issue of the EEC's Joint European Torus (JET) fusion project blew up again, as it was always destined to do. JET itself has not yet blown up, and for many that is some comfort. But the possibility nevertheless remains, and the absence of a decision on a site for the project, which is and always has been the crucial issue, now symbolically represents all the current problems of cooperation that the EEC would dearly like to avoid.

The background is familiar. Of the original sites proposed for the project, four stood out: the EEC's research establishment at Ispra in Italy; Cadarache in France; Garching in Germany; and Culham in Britain. Italy held out for Ispra until its future was more or less guaranteed through judicious deployment of the EEC's joint research programme for 1977-80. France held out for Cadarache, with CERN as an alternative, until it could do so no longer—which was effectively until it could secure a better deal to present back home. That left Garching and Culham.

The character of that 'deal' is not so familiar. But it was crucial to the failure of the 29 March meeting of the Council of Research Ministers at which the whole matter was to have been finalised. No one talks about deals in the 'open' Community, so even the most popular theories are speculative. But basically the idea seemed to be that Britain would at last have the Community project it wanted by receiving JET, that the project director would be German, and that France's proposed management structure would be used. Agreement would then have been possible on a majority vote.

But something went wrong. The small countries, already apparently miffed at the failure to agree on farm prices earlier that week, objected to the ostensible collusion. The majority shifted and the arrangement, inasmuch as it existed at all, began to fold. Details of the proposed management structure, which apparently involved the creation of a JET board of managers with a degree of detachment from the European Commission, also became an issue, making a decision on the site seem pointless.

At the same time, however, final

approval was somewhat surprisingly given in a separate vote to the joint research programme. Until then the issue was inextricably linked to the matter of the JET site; now the link seemed broken. In fact it wasn't, because there was still the all-important matter of releasing the agreed funds for the programme. This Britain has since refused to do, maintaining that it got its fingers badly burned in March when, in the Energy Council meeting on the same day as the Research Council, its concessions on the Euratom loans scheme unexpectedly failed to yield agreement on a minimum safeguard price for oil. Agreement on that issue had been expected in turn to encourage the intended agreement involving JET and the research programme.

All this left the Community with a need both to finalise details about the management of JET and, as usual, to try to reach an understanding on the site before the next Research Council. Meetings of officials proceeded accordingly. But no meeting of the council was set for before the end of June because it was only then that Britain's chairmanship, by now under suspicion, was due to give way to Belgium's. That spelled trouble, because contracts on which the JET design team at Culham were working were also due to expire at the end of June.

Although the Commission was given the authority in March to extend the project beyond June, and presumably has whatever money it needs to do so (the team's salaries are in fact paid by their own home laboratories), a decision on the future of JET before the end of June could only come in a meeting of the Council of Foreign Ministers which was due this week, on 21 June, or next week, at the heads of government meeting on 29 June. Hence all the activity last week, even though JET was not the business of the Energy Council, nor, strictly speaking, the regular business of the foreign ministers or prime ministers.

The sudden burst was typical of the way the Community conducts its business. Guido Brunner, the Energy and Research Commissioner, on the day before the Energy Council, warned that the absence of a decision before the summer recess—at the Foreign Council or heads of government meeting, in other words—would amount to an abandonment of the

project. He was responding to a telegram received from the JET design team expressing concern over the problem; he had also reportedly received a telegram from Ispra staff complaining about the hold-up in research programme funds which they thought was threatening their jobs.

Then JET came up as a spin-off from the Energy Council when at a press conference afterwards the UK minister Dr Dickson Mabon reminded people of Britain's strong desire to have JET. A parliamentary question in the House of Commons later produced the answer that, although remaining differences had recently been narrowed, if discussion was still in progress by the end of June, "we hope the Commission will again extend the contracts of employment of the design team".

On top of this came allegations in the European Parliament, which were spurned by the UK official there, that Britain's hold-up of research funds amounted to blackmail. The official had admitted publicly that two separate issues were intimately tied together as a political package. But he assured listeners that there was enough money for the research centres to continue work and pay salaries until the autumn.

Then later last week came reports that behind-the-scenes contacts were under way between London, Brussels and Bonn involving third party mediation, perhaps from the next country to hold the chair, Belgium, in an effort to secure a solution before the end of the month.

That must be the fervent hope of all those who want to see the project survive. Yet it remains that the lack of trust Britain has felt over JET since the March débâcle is now almost fully reciprocated by other member states on this as well as other issues. Both sides now want impossible guarantees of a *quid pro quo*, and refuse to move until the other does.

Meanwhile a date for the next Research Council had at the end of last week still to be set. If nothing is achieved by the end of the month, then what is called an "orderly winding-down" of the project will begin. In other words, like the other European project, Dragon, which died last year in such ignominious fashion, JET too could be cancelled for lack of interest.

Chris Sherwell

COMECON

● The recent concern in the West about the dangers of nuclear power evoked a uniform and perhaps predictable response from the Comecon media; such fears, it is alleged, are the result of political scare-mongering and/or capitalist negligence. The nuclear power plants that are designed and built in the Comecon bloc on the basis of Soviet R&D, it is claimed, constitute no hazard to health or the environment.

This confidence, although it may be excellent propaganda, hardly constitutes a sound basis for planning an energy policy which is heavily committed to a change-over to nuclear generation as rapidly as construction and logistical problems permit. (It is estimated that by 1980, some 10% of the generating capacity of the whole Comecon bloc, including Cuba and Mongolia, will be nuclear.)

The risk of accidental pollution, however, is appreciated by the planners. When the Institute of Radioecology and Nuclear Technology was founded two years ago at Kosice, Czechoslovakia, systematic research was begun, not only into the prevention of accidental escapes of radioactivity, but also into the ecology of living organisms in an environment polluted by radioactive waste. According to Milan Zaduba, the Director of the Institute, this is the only research institute in the world making such a study. This unique position, however, will not be maintained for long, since future plans for the institute envisage the gradual transformation of its activity into a programme of multinational cooperation with institutes in the Soviet Union, Poland, and the GDR.

● Bulgaria, as one of the less-developed members of Comecon, has a science and technology programme which is closely coordinated to that of the Soviet Union. At present, there are more than 30 agreements between the two countries involving 17 Bulgarian and 48 Soviet ministries and 20 bilateral working groups. To date, the flow of information has been largely in one direction, the Soviet Union having supplied Bulgaria with two-thirds of her machinery, equipment and complete plants, and with over 6,500 complete sets of technical and scientific documentation.

The flow of information, however, is now becoming somewhat more balanced. Uses are being found for Bulgarian technical documentation and a number of Bulgarian innovations are being introduced into

various branches of Soviet industry. Recently, a joint Soviet-Bulgarian Institute, Interprograma, was founded in Sofia, to develop software for automated control systems for production and management. The name of the institute is reminiscent of numerous Comecon projects and co-



ordinating centres, such as Interkosmos and Interatominstrument, and suggests that the new institute may possibly be expanded, in the future, into a similar multinational body.

The same possibility is also suggested by the recent agreement setting up Intervodochistka, the Comecon international R&D corporation dealing with problems of water conservancy and purification. Under this agreement, Bulgaria is charged with producing the various digital automated units which the corporation will need. From the communique issued by the 80th session of the Comecon Executive Committee in April, it would appear that Bulgaria's responsibility will be extended to providing similar equipment for other branches of the economy.

● Poland's inland waterways have been a matter for concern over a number of years, but it was not until January 1976 that a government decision finally settled the long-standing debate as to whether the waterways were worth modernising and developing at all. By this time, a number of weirs and locks had become urgently in need of repair, but in some cases, the conflicting demands of other sectors (notably the Ministry of Agriculture) have meant that repair work has had to be abandoned. Moreover, during the years of neglect, the waterways had become a convenient means of dumping industrial effluent and sewage, and now only 53% of effluent is treated at all,

the rest being allowed to discharge into rivers and lakes. Some 28% of the total length of the rivers is said to be "beyond economic utilisation".

At present, water consumption in Poland is 13,000 million cubic metres per annum, and is expected to rise to 18,000 million cubic metres in the next few years. While the civil engineers are struggling to produce a rational scheme for the renovation of river installations to cope with modern traffic, plans are also being discussed for the reduction of pollution. Suggested remedies include the use of more easily soluble detergents and pesticides, the recycling of agricultural effluent, and the development of water-saving technologies. One factory, the new Gorazdze cement works in Opole province, however, seems unlikely to add to the pollution problem. Although this plant is sited near the Odra, it needs the river as a means of transport only; the raw materials are prepared by a dry pulverisation process. "The advantages are obvious," commented *Dziennik Ludowy*. "Where there is no water, there is no effluent."

● According to Yu. Maksarev, the Chairman of the State Committee of the Soviet Union on Inventions and Discoveries, one in three of all patent applications in the world is made in the socialist countries. To cope with this vast flow of invention, the Comecon countries are at present engaged on working out a system to improve the national patent information systems of the member countries and also, by means of "division of labour, cooperation, and the centralisation of individual information processes", to increase the volume of information exchanged between member countries.

According to Maksarev, invention in the Comecon member countries is based on a study of world patent information. This study has not, it would seem, had any marked effect on the type of information patented by the Soviet Union, which, with some 100,000 'author's certificates' published in the last three years, is responsible for the majority of Comecon inventiveness. A close study of these 'certificates' reveals that in a significant number of cases, the novelty would be insufficient for a patent to be granted in any Western country, being at best the type of innovation which would be registered as a 'utility', and at worst simply minor modification which, in the West, would be incorporated as an unregistered factory improvement.

Vera Rich

WINDSCALE

Down to business

The Windscale inquiry opened in Whitehaven, Cumbria, last week. Chris Sherwell reports

NO ONE thinks a decision on Windscale is going to be easy; the case is too finely balanced. But practically everyone involved in the inquiry, on the application from British Nuclear Fuels Ltd (BNFL) to develop land there for an oxide fuel reprocessing plant, hopes that the decision will go their way. Once Mr Justice Parker has made his recommendations, probably by the autumn, the responsibility will lie with the Secretary of State for the Environment. Assuming the Labour government survives until then, that will be Mr Peter Shore.

Opening statements from BNFL and from the various opponents of the project were heard last week. Cross-examination of some 18 witnesses to be brought by BNFL also began. The first was Mr Con Allday, the managing director, and he was still on the stand on Friday. Before he appeared the inquiry already looked like broadening the spread of issues it will cover when Mr Justice Parker produced over a dozen points on which he said he and his two assessors wanted early clarification from BNFL.

The points drew attention to figures showing that plutonium recovered through reprocessing would be enough to charge more fast breeder reactors than the country could possibly need. Mr Justice Parker said they wanted to know if one objective was to keep the breeder option open. They also wanted to know how much plutonium was required to fuel a breeder and how much had been recovered and would be recovered from spent Magnox fuel.

The implication was that existing facilities at Windscale for reprocessing oxide fuel—using the 'head-end' plant which is presently inoperative—might cope with domestic reprocessing demands and take some overseas business, and with other work also satisfy Britain's plutonium requirements. In short, was a new plant necessary?

This all fitted with what Mr Justice Parker had said at the start of the whole proceedings about three basic questions needing answers. These were: should spent oxide fuel be reprocessed in Britain at all? If yes, should it be reprocessed at Windscale? If yes, should the plant be larger than that required for domestic purposes in order to reprocess fuel from abroad?

In the context of these questions it

was hardly surprising that the precise terms of the Japanese contract that BNFL has been trying over many months to negotiate also became important soon after Mr Allday took the stand. He was reluctant to furnish the document, yet plainly, without the terms of the proposed contract, no detailed assessment could be made of the financial and economic factors which, for the third question at least, BNFL themselves must regard as relevant. Sight of the terms was subsequently allowed.

Under cross-examination Mr Allday admitted it would be cheaper to use refurbished or rebuilt facilities that existed, but said this would not be enough. He also endeavoured to explain why existing facilities to reprocess spent Magnox fuel from Britain's first generation reactors had failed to operate at full design capacity. He attributed this at least partly to the difficulty of handling spent fuel elements that were in a poor condition because they had stayed in reactors too long. Also broached was the question of what work was being done on dealing with fuel if it should not be reprocessed.

Earlier in the week the inquiry had opened amid a burst of publicity generated by the presence in Whitehaven of nuclear experts, company men, government officials, lawyers, environmentalists, demonstrators and journalists. Many detected a note of resentment among the local people who have to live with Windscale. But the resentment was less at the plant, expansion of which means more jobs, than at the foreign invasion. Non-BNFL voices supporting the project because of the benefits it would bring to the area came from Cumbria County Council and Copeland Borough Council. But another authority, the Isle of Man, is among the objectors, not all of whom agree on the basis from which their arguments can best be launched.

Before the inquiry opened BNFL confirmed that a laboratory at Windscale had been closed following an accident six weeks earlier in which a pressurised glove-box had blown back and released plutonium. During the week, as part of a new policy of directly releasing details of all accidents no matter how trivial, BNFL published a list of incidents that had occurred over the previous two months. These details are usually passed on to the Nuclear Installations Inspectorate and to the Secretary of State for Energy.

The inquiry continued this week with more evidence from Mr Allday. Other BNFL witnesses were also to appear. □

URANIUM

Hot topic

A TEAM of experts from Australia is thought to be in Europe to discuss possible sales of uranium for EEC nuclear power stations. They were expected to visit Brussels to discuss marketing arrangements and review Euratom security controls. The Australian Prime Minister, Mr Malcolm Fraser, visited Brussels last week for talks with the European Commission. He told reporters afterwards that his government had not yet decided on its export policy for uranium. An announcement is expected within weeks now that the second report of the Fox Commission has been published.

In spite of speculation to the contrary, Mr Fraser said that there was no question of a trade-off between possible exports of Australian uranium and greater access to EEC markets for Australian agricultural produce. Overall commercial relations between Australia and the EEC can, however, be expected to influence the matter, and Mr Fraser has attacked the EEC's Common Agricultural Policy.

Mr Fraser would not answer questions about Australia's alleged membership of a world-wide uranium cartel, into which further probings were made in the United States last week when a bundle of internal memoranda of the Gulf Oil Corporation was released by a Congressional subcommittee. They seriously undermine Gulf's contention that it was an unwilling partner in the cartel, and contradict its assertion that the operation of the cartel had no effect on domestic uranium prices in the United States. The memoranda, which were supplemented by a three-and-a-half hour grilling of Gulf's chairman, Jerry McAfee, could pose serious problems for Gulf's defence against possible anti-trust suits.

McAfee persistently claimed at the hearing that Gulf's Canadian mining subsidiary was pushed into the cartel by the Canadian government. He did not give details, however, and some of the internal memoranda depict the company as a willing partner. One memorandum even discussed how the cartel could eliminate competition from other uranium companies. The cartel was formed in 1972, and operated until 1974, a period when uranium prices increased sharply. McAfee and other Gulf officials admitted in their testimony that the increasing world price of uranium had, to a limited extent, also driven up domestic prices in the United States.

The second annual meeting of the Uranium Institute was meanwhile due to take place in London this week. The institute, established in June 1975, exists "to promote discussion between uranium producers and consumers", particularly on uranium supply and demand. It has 36 members, including 21 producers and 9 consumers. There are no US members. The institute's secretary general, Mr Terry Price, was among those called to give evidence before a US judge at the US embassy in London last week in connection with a case brought by Westinghouse Electric Corporation concerning the cartel.

IN BRIEF

Petition to Poles

Three Nobel prize-winners—George Wald (medicine), William Lipscomb (chemistry) and Kenneth Arrow (economics)—and some twelve other well-known American scientists are among the 139 signatories of a petition to the Polish government asking for the immediate release of the imprisoned members of the Workers' Defence Committee "whose only crime consists of defending the basic human rights of others".

Meanwhile it is reported from Moscow that the physicist Professor Mark Azbel has, exactly six years after his first application, been granted permission to emigrate to Israel. Since 1974, when Professor Alexander Voronel went to Israel, Azbel has been chief organiser of the illicit Sunday Seminars for *refusnik* scientists.

UK joins in on test ban

The Soviet Union and United States began talks in Washington last week on how to achieve a comprehensive nuclear test ban. The Russians are reported to be keen to come to some agreement on a test ban and President Carter is said to have made it a priority in his programme of arms control.

As the result of a Russian request Britain will join the two super powers, starting 13 July, in drawing up a comprehensive nuclear test ban treaty. The three countries have collaborated before on nuclear arms control: in 1963 on the partial test ban treaty and in 1968 on the non-proliferation treaty. It is thought likely that Britain will go along with a comprehensive treaty provided that it includes nuclear explosions for peaceful as well as military purposes.

Neutron source

Rutherford Laboratory has been given the go-ahead to build a spallation neutron source. The announcement, during the official opening of the laboratory's laser facility (see page 666), greatly eases the transition of the Rutherford into a new era.

Nimrod, the laboratory's 7 GeV proton accelerator, is due to close in 1978, and although the new laser facility goes some way towards using the laboratory's expertise, there had been fears that in the absence of further projects there would have to be redundancies. The spallation neutron source, however, in which 800 MeV protons pulsed at 50 cycles per second will be fired at a heavy element sources to break up nuclei and yield fast neutrons, will use much of the existing human and hardware investment in Nimrod.

I recently attended the 1977 Sponsored Film Festival, organised by the British Industrial and Scientific Film Association and held in London during ten days in May. During that period 188 films and video tapes were shown, and numbers of prizes were awarded by distinguished panels of judges. The films were divided into ten categories—education, training, safety, technical, scientific and research and so on. I restricted my attendance to "Ecology and the Environment".

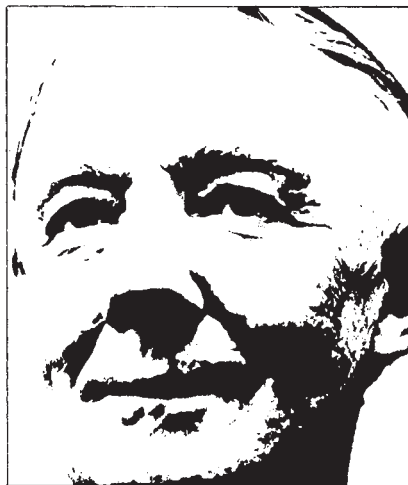
This was an interesting, and, to me, a new experience. Although in my youth I was an enthusiastic film goer, witnessing the change from the silent film to the 'talkie', and the peak of the industry's prosperity, I have not been inside a commercial cinema for several years. I occasionally watch old feature films on television, and have often seen, and used, short scientific films at lectures and conferences. I have even been concerned with making some such films. But I have never before sat from 9.30 am to after 5 pm watching a continuous series of moving pictures.

I found that the organisers of the festival had given our theme a wide interpretation, for in addition to seeing productions about energy and pollution, nature reserves and the protection of wildlife, we were also treated to propaganda films trying to persuade us to visit Guernsey, the Lake District or the historic castles of England and Wales.

In one way or another, the oil industry was prominent. The government's Central Office of Information screened "Clean and Pleasant Land" showing the horrors of oil pollution on the sea and the beach, and how

Britain was coping with the problems. Two potential polluters, British Petroleum and Shell UK brightened their tarnished images with an admirable sermon on energy saving and a pic-

Ecology on film



KENNETH MELLANBY

ture of the, as yet, unspoiled Peak District National Park.

Most of the rest of the programme was more truly environmental and ecological. The Royal Society for the Protection of Birds, Britain's largest voluntary conservation organisation, showed two films made by their own unit. In the first, David Bellamy, one of our leading telly-dons, described the dangers to our small areas of surviving heathland. In the second, Ian Prestt, the Society's Director, spoke persuasively about its work. These combined propaganda with education, and included good and unusual natural history photography.

However, as I watched, I wondered if some films might not, by their very excellence, defeat their own objectives. For instance "The Petersfinger Cuckoos" enabled the cinema audience, and those who watched it on television, to observe the most intimate details of the private life of this elusive and parasitic bird. It all looked so easy, yet we know that "the few seconds of the secretive cuckoo laying its egg took more than 1,000 hours of waiting and watching". I have sometimes been worried lest inexperienced town-dwellers who have watched such programmes may be discouraged when they visit the country and see comparatively little wildlife, even on a nature reserve with the help of a skilled naturalist.

Fortunately, this does not seem to be the case. The sound of the voice of a real, wild, live cuckoo seems to be appreciated all the more. When, at our home in Huntingdonshire, we open our rural and unkempt English garden to the public, to raise money for various worthy conservationist causes, we set out a simple nature trail demonstrating wild flowers ('weeds' to the conventional gardener), birds' nests and even newly-dropped fox dung. We find that most of our visitors, and all of their children, appear to be enthralled. It seems that the most sophisticated nature films are treated as something different from real life, in the same way that violence in Westerns and crime programmes seems unrelated to that experienced in our streets and after football matches. Nature films are undoubtedly useful, but they are no substitute for the real thing.

correspondence

Unemployed scientists

SIR,—Your article (21 April, page 668) describing the plight of Soviet scientists who have been refused employment and have tried to carry on their work in isolation rang a familiar bell. But, although the world receives news of these unhappy people in the Socialist countries, no mention is made of the plight of hundreds of unemployed biologists, chemists, physicists, and mathematicians in the Free World.

Any science department in Canada which ventures to advertise a vacancy gets snowed under by anywhere from 75–400 applicants most of whom are underemployed and receive no professional recognition from their colleagues. Despite this, universities go on pretending that no such problem exists, and have not taken steps to expand their faculty to alleviate it. The unemployed scientist has no faculty union, cannot apply for research grants, cannot receive travel grants, and must work in isolation. He is barred from receiving unemployment assistance unless willing to seek any type of work, thereby relegating his professional career, for which he and his parents have paid dearly, to the status of a part-time hobby.

Every government adopts certain policies for eliminating its unwanted and superfluous citizens. The methods of the Socialist and Free-World countries differ only in superficial aspects. A doctoral student whom I taught at the University of Calgary sought suitable employment for five and a half years from 1968–73, before taking his life, leaving a widow and three small children. Can we truly say that such people have suffered any less than the 'refusniks' of the Soviet Union?

Yours faithfully,

JAMES J. KLEIN

University of Regina,
Saskatchewan, Canada

Let n be the psammit

SIR,—What is the quantity of which the mole is the SI unit? It is 'amount of substance' (in French *quantité de matière*). However, Newton's definition of mass was as quantity of matter (in Latin *quantitas materiae*), and the word 'substance' carries overtones of theology and mediaeval philosophy. It is only necessary to recall the snickers

among scientists when 'amount of substance' was introduced, or the bewilderment of students who try to make sense of the expression, to realise that 'amount of substance' does not describe what it names, and that English needs a simple word for this quantity, as German has *Stoffmenge*.

Common speech does not have a word that can readily be extended to cover the concept, but several classical roots might yield a suitable word. Although grains of sand had been a Greek metaphor for uncountability, Archimedes in his *Sand-Reckoner* (*Psammités* in Greek) calculated the number of grains of sand that would fill the known universe. To replace 'amount of substance,' I propose 'psammit', evoking Archimedes' calculation and treated as if there were a Latin *psammitia* from which it derives, with combining form 'psammito-' and adjective 'psammitic'.

How would these be used? It is usual to write, "Let m be the mass . . .", and only later need it be revealed that the unit will be the kilogramme. On the other hand, at present we write, "Let n be the number of moles . . .". If instead we write, "Let n be the psammit . . .", the dimension of n is clear, but the unit, whether mole, micromole, or pound-mole, need only be specified when numerical values are given. Again, when the density of a gas is expressed in moles per unit volume we must at present speak of molar density, but this would become psammitic density. Other uses of psammit follow by simple analogy with mass and length, where common usage already distinguishes the quantity from the unit.

Yours faithfully,

GEORGE S. KELL

National Research Council,
Ottawa, Canada

Microprocessor myths

SIR,—I fear that some of the 'myths' mentioned in Basil Zacharov's article on microprocessors (28 April, page 760) were actually fluffy and insubstantial.

It is true that microcomputers cost more than microprocessor chips; more, even, than naked minis. But what is of genuine importance is this. One can now buy—and pay for—only as little computing power as one actually needs, right down to a four function calculator costing \$7. No longer must one

spend the \$7 every half hour in connection charges, and waste time keeping up on the latest system releases, just to be able to calculate small profundities on demand.

Microcomputer users may also spend inordinate amounts of time writing software, but after all, anyone making many small-scale runs, rather than engaging in massive, production number-crunching, must write a seemingly inordinate quantity of software (or spend almost as much time learning to use canned software). The microcomputer also has one overwhelming advantage with respect to the software investment: ownership and control. That control provides a modicum of assurance that one's heavy investment in software will not suddenly be destroyed or devalued at the whim of an outside party. The real question is: how can anyone afford not to use a computer small enough to own, whenever possible?

It is also true that microcomputers are not fast, complicated, and sophisticated like minis and mainframes, but the power of a pocket calculator is sufficient for a large class of tasks. A microcomputer system costing a mere \$1,700 is likely to be severely limited by the user's communication speed.

None of this is new. After all, that 'complex internal organisation' which allows a big mainframe to perform 'many concurrent activities' is customarily installed precisely so that the mainframe can simulate a hundred or a thousand small computers running at once, each serving a different user. Today, we can sometimes spare ourselves that nonsense, and simply purchase the hundred computers.

Small computing tasks have tended to be served inconveniently, expensively, and sometimes even with grudging arrogance, by traditional computer facilities. Microcomputers and LSI technology complete the low end of the continuum of computer powers. It should, therefore, not be hard to understand the microprocessor phenomenon. After all, it is practically one and the same with the calculator revolution. Even diehard advocates of mainframing everything have been known to use calculators.

Yours faithfully,

PAUL SCHICK

University of Wisconsin-Madison,
USA

news and views

New laser facility at Rutherford

from T. P. Hughes

THE Science Research Council's Central Laser Facility at the Rutherford Laboratory was officially inaugurated on 20 June. The occasion marks the successful completion and commissioning of the most powerful pulsed laser in the United Kingdom, whose output is available to University research workers for a variety of experiments.

The original proposal for a central facility came from several University groups investigating aspects of laser-plasma interactions. The experimental groups had previously built their own high-power lasers, but needed even higher powers to extend their work to higher plasma temperatures and densities and laser irradiances. They were acutely aware not only of the high cost of large lasers, but also of the difficulties of maintaining a reliable output from home-made systems, whose servicing occupied a disproportionate amount of time at the expense of the interaction experiments themselves. After extensive discussions, it was decided to ask the SRC to provide and operate a large neodymium laser, and to make funds available to update it and, in due course, to replace it with another laser as techniques develop.

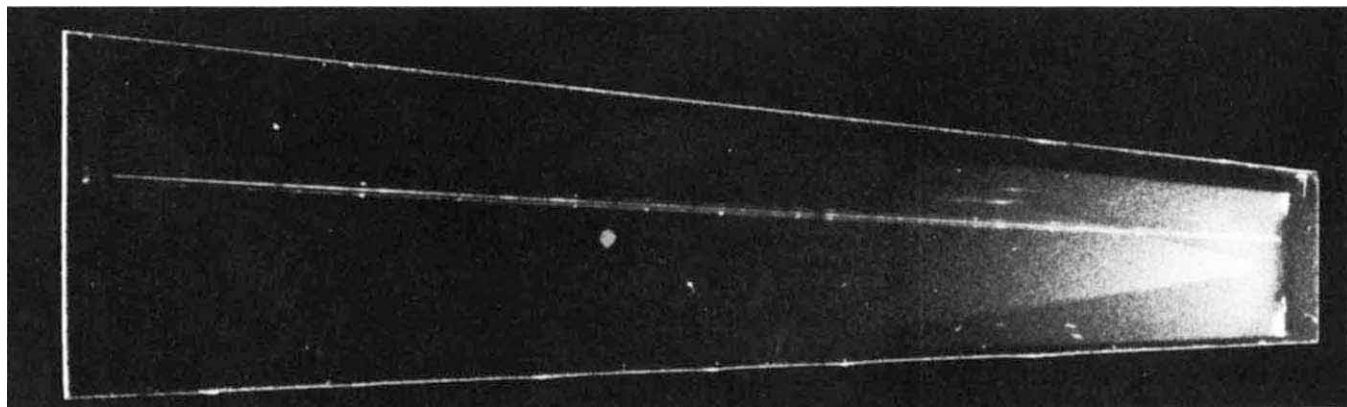
The scientific objectives of the Laser Facility are to create and study dense plasmas generated by the laser compression of matter; to study inter-

actions of intense laser radiation with matter; and to develop new and more efficient high-power lasers for these and other purposes. The creation of very dense plasmas by ablative compression of small isolated targets is currently being investigated in several laboratories throughout the world as a potential route to controlled nuclear fusion, but it has a much wider scientific significance. It is now possible to produce plasmas in the laboratory with temperatures and densities similar to those which are believed to occur in stellar interiors. Information about spectral line shapes and intensities, about radiation and other energy transport processes, and about the equation of state in these plasmas will be of great value to astrophysicists. Several other experiments using the laser facility were also envisaged in the original proposals, including the development of VUV (vacuum ultraviolet) and X-ray lasers, studies of gas breakdown and experiments in quantum electrodynamics.

Experimental programme

At a meeting of prospective users from the Universities and other laboratories in December 1975 the basis of the initial experimental programme was agreed. Detailed proposals were prepared for single-beam experiments to begin when the "driver"

stage of the neodymium laser was ready, with an output of up to 10 J in a 100 ps pulse, and for two-beam experiments using the full laser system to compress glass microballoons. During the following 12 months groups of scientists, each drawn from several universities and other laboratories, collaborated with the staff of the newly-formed Laser Division of the Rutherford Laboratory to design and construct a large target chamber and a wide variety of experimental equipment. In the meantime, the laser was being installed and tested. The first single-beam experiments began in December 1976 and continued until the end of March 1977, with several pauses to permit further work on the laser. During this period many plasma diagnostic techniques were tested and some valuable X-ray and VUV spectroscopic data were recorded, including information relevant to the interpretation of solar flare spectra. Preliminary observations of the time required for the laser pulse to burn through thin foil targets, prepared at the Rutherford Laboratory, were compared with computer calculations to obtain information about the thermal conductivity of the plasma layer. The two-beam laser system was completed in April 1977; the first shot at a microballoon target produced compression, as recorded with an



The illustration shows a space-resolved X-ray spectrogram from laser-compressed neon-filled glass microballoon.

X-ray pinhole camera. In this experiment the glass microballoon, 85 μm in diameter and with walls 0.5 μm thick, received only a tenth of the full rated power of the laser, focused with two $f/1$ lenses. Further experiments using microballoons selected, mounted and filled with gases such as neon and argon at the Rutherford Laboratory are now in progress (see illustration).

The laser pulse is formed initially by switching out a single spike from the mode-locked output of a neodymium-YAG oscillator. Its duration may be varied between 30 and 300 ps. A series of neodymium glass rod amplifiers of increasing diameter, interspersed with Faraday isolators and spatial filters, produces an output pulse from the driver stage with an energy of up to 10 J in a single beam of 76 mm diameter. The beam is then divided into two parts, each of which passes through a further rod amplifier into a 10 cm diameter disk amplifier to give a rated output of 40 J, corresponding to a total output of 0.8 TW in a 100 ps pulse. For stable operation and to prevent damage from dust particles, the laser is housed in an air-conditioned clean room: the beams emerge through ports in a partition into a separate target area. Here beam steering reflectors direct the laser pulses into the main target chamber. This chamber, which is evacuated, has more than 40 ports for windows and for diagnostic equipment. Within it, attached directly to the laboratory floor and isolated from the vacuum envelope by bellows, is a massive optical table on which are mounted the target holder and the two large aspheric focusing lenses. Focusing adjustments are made with the aid of a continuous wave Nd-YAG laser and a TV telemicroscope: the peak irradiance in the focal spot due to a single beam is in the region of 10^{15} – 10^{16} W cm^{-2} . The laser is controlled from another room which also houses recording oscilloscopes and a small computer used to monitor the laser and to acquire and process experimental data.

More than 30 University research workers from eight Universities are at present participating directly in the experimental programme. Theoretical and computational work on related topics, including notably the hydrodynamics of the compression of spherical targets, is also in progress at five Universities. For many of those involved in the experiments, accustomed to working in comparative isolation, collaborative research is a new and stimulating experience, which imposes its own discipline on their

activities in exchange for access to the best available research equipment, and for the rapid progress which is possible when new results and ideas are immediately and freely shared. The Rutherford Laboratory has, of course, been running collaborative research programmes, notably in high energy physics, for many years, and provides excellent facilities for visiting scientists.

The present programme of research will continue well into 1978. More sophisticated focusing optics, providing more uniform illumination of spherical targets, will be supplied shortly. New experiments on X-ray and VUV lasers are currently being planned. The rate of progress is limited by the availability of laser shots, and this situation is likely to persist into the foreseeable future: the Laser Facility is already as heavily loaded as staffing permits. Nevertheless, proposals for appropriate new experiments will always be welcomed. $\square$

Neutron scattering and ribosomes

from Richard Brimacombe

PHYSICAL chemistry has had a difficult time in coming to grips with the ribosome; in the past, all too many painstaking studies have led only to rather unsatisfactory conclusions, usually involving unspecifiable conformational changes or else bemoaning the complexity of intra-ribosomal interactions. This is however by no means the case with neutron scattering measurements, which are beginning to give fairly precise estimates of useful intra-ribosomal distances, the most recent contribution being a paper by P. B. Moore, J. A. Langer, B. P. Schoenborn and D. M. Engelmann (*J. molec. Biol.* **112**, 199; 1977), entitled 'Triangulation of proteins in the 30S ribosomal subunit of *E. coli*'.

Neutron scattering has already been applied to the *Escherichia coli* ribosome to measure the distance between the centres of mass of protein and RNA within the ribosomal subunits, and here it appears that in the 30S subunit, centres of mass are more or less coincident (Moore *et al. J. molec. Biol.* **91**, 101; 1975). On the other hand, in the 50S subunit they are separated by a significant distance, indicating that the large subunit has a protein-rich and RNA-rich region, although there is some disagreement as to the magnitude of the displacement (Moore *et al. Proc. natn. Acad. Sci. U.S.A.* **71**, 172; 1974; Serdyuk & Grenader *FEBS Lett.* **59**, 133; 1975;

Stuhrmann *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2379; 1976).

Similar neutron scattering studies are currently being made in a number of biochemical systems (for investigating chromatin and virus structure, for example) but the advantage of using neutron scattering to investigate the *E. coli* ribosome is that active ribosomal subunits can be re-assembled from their constituent RNA and proteins, which enables a reconstituted particle to be prepared in which selected pairs of ribosomal proteins are deuterated. The neutron technique not only allows the distance between the centres of mass of the two deuterated proteins to be measured, but also gives some information about the shapes of the proteins concerned. In the most recent paper by Moore *et al. (op. cit.)* six such inter-protein distances are described, which, when added to those previously published by the same group (Engelman *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3888; 1975), gives a total of nine inter-protein distances within the 30S subunit. These are between proteins S2–S5, S3–S4, S3–S5, S3–S7, S3–S8, S4–S7, S4–S8, S5–S6, S5–S8, and the authors are able to use their data to construct a map of the relative locations of the centres of mass of S3, S4, S5, S7 and S8, in which only two distances need still to be measured in order to determine the relative placements of all five of these proteins in three dimensions.

Moore *et al.* conclude that most if not all of the proteins under study have significantly elongated conformations, with protein S7 being particularly oddly shaped. This finding is in full agreement with a number of recent studies, notably small-angle X-ray scattering of isolated proteins (for example Osterberg *et al. FEBS Lett.* **73**, 25; 1977), and the mapping of antigenic determinants on the ribosome surface by immune electron microscopy (reviewed by Stöffler & Wittmann in *Protein Synthesis* (eds Weissbach & Pestka) 117–202 (Academic Press, New York, 1977)), which also indicate that many ribosomal proteins are highly extended.

Similar measurements by neutron scattering are also currently being made on the 50S subunit; one inter-protein distance (between L7/L12 and L10) has already been published (Hoppe *et al. in Brookhaven Symp.* No. 27, IV 38; 1975), and measurement of several others is in progress. It is clear that the neutron technique has a useful future in ribosome research, and as measurements continue to be made the method will (as Moore *et al.* point out) reveal its own degree of

T. P. Hughes is a Senior Lecturer in the Department of Physics at the University of Essex and a Consultant to the Laser Division of the Rutherford Laboratory.

Richard Brimacombe is in Dr Wittmann's Laboratory at the Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem.

self-consistency; within the three-dimensional lattice, each new distance serves as a quantitative check on some of those already measured.

The elongated nature of the proteins makes it rather difficult to compare the neutron results in any detail with other data, such as protein-protein cross-linking results, or the immune electron microscopy technique mentioned above. There is however another physical technique which gives data very similar to those obtained by neutron scattering, and this is the measurement of energy transfer between fluorescent dyes (Huang *et al. J. molec. Biol.* **97**, 443; 1975). Here the ribosome is re-assembled with two specific proteins labelled with two different fluorescent dyes, and the energy exchange between them can be measured in the reconstituted particle. Making certain assumptions, this gives an estimate of the relative distance between the centres of mass of the proteins concerned (although without giving any information on shape), but unfortunately the set of protein pairs which have been determined by this latter method does not overlap sufficiently with the set measured by neutron scattering for a direct comparison to be made. It is however obviously only a matter of time before the two sets of data do begin to overlap, and that should be something well worth waiting for. □

Surface antigens specified by the *T/t* locus

from P. L. Stern

THE idea that molecules displayed on the cell surface may allow differential recognition of interacting cells and therefore be a key factor in morphogenesis is an extremely attractive one. An important corollary to this view is that at least some genes which play an essential part in development would be expected to specify components of the cell surface. So recent observations suggesting that early embryonic cell surface antigens are specified by alleles at the *T/t* locus in the mouse have aroused a great deal of interest. Mutants in this locus affect embryonic development, sperm production and function and genetic recombination (see review by Bennett *Cell* **6**, 441; 1975). Many of these *t* mutants are embryonic lethals when homozygous and

seem to affect development at very specific stages.

In 1970 Gluecksohn-Waelsch and Erickson put forward the hypothesis (*Current Topics Devl Biol.* **5**, 281) that *T/t* products may be expressed on the cell surface and that the defects in the mutant embryos were due to malfunctions in cell-cell recognition and morphogenetic movements. Two lines of evidence from serological studies have suggested that the products of the *T/t* locus are indeed expressed at the cell surface although it has not yet proved possible to identify unequivocally any surface component as a product of any *t* allele.

One of the first candidates for a *t* locus gene product was the component bearing the F9 antigen. F9 antigen(s) is detected by an antiserum prepared by multiple immunisations of syngeneic mice with irradiated F9 embryonal carcinoma cells (Artzt *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 2988; 1973). F9 antigen is not expressed by any adult tissues except sperm and testes germ line derivatives but is expressed in normal embryos. The fertilised egg has no detectable F9 antigen expression but expression increases during cleavage up to the morula stage and can be detected until day 10 of embryonic development (Jacob *Immun. Rev.* **33**, 5; 1977).

This antigen, like the *T/t* locus effects, is expressed in embryonic cells and sperm. It therefore became a candidate for the product of an early acting wild-type allele at this locus, namely t^{w32} (t^{12}). If the t^{w32} mutation were affecting the expression of F9 antigen, twice as many sperm from heterozygous $t^{w32}/+$ t^{w32} animals as for wild type $+/+$ t^{w32} should be required to remove a given anti-F9 activity, provided that sperm cells express each allele carried by the animal. This result was obtained by Artzt *et al. (Proc. natn. Acad. Sci. U.S.A.* **71**, 811; 1974) using quantitative absorption of anti-F9 serum; t^{w1} and *T* heterozygote sperm exhibited wild-type absorptive capacity. The interpretation that F9 antigen is specified by a wild-type allele at the t^{w32} locus is, however, made difficult by the fact that some recessive *t* lethals significantly suppress crossing over for a considerable length of chromosome 17. Hammerberg and Klein (*Nature* **253**, 137; 1975) have shown by typing H-2-associated antigens of mice carrying recessive *t* alleles, that particular *t* factors (within the same complementation group) represent a certain combination of genes in chromosome 17, a *t* haplotype, which has remained genetically interlocked in mouse populations for many generations. In consequence the differential absorptive capacity of the heterozygous sperm from wild type could reflect the activity of genes anywhere within the region of crossover suppression.

If anti-F9 serum detects the $+/t^{w32}$ gene product then this gene product

might be altered or absent from t^{w32}/t^{w32} embryos although not from other t^x/t^x embryos. Kemler *et al. (Proc. natn. Acad. Sci. U.S.A.* **73**, 4080; 1976) examined the progeny of crosses producing a proportion of embryos homozygous for t^{w32} , t^{w5} , t^{w18} and *T*. In crosses producing t^{w18} or *T* homozygotes, anti-F9 serum labelled nearly all the embryos similarly to normal morulae. Among the progeny of crosses producing t^{w32}/t^{w32} embryos, a significant proportion were not labelled. Further, the embryos lacking F9 were blocked in their development at the morula stage at which t^{w32} arrests development. However when the progeny of a balanced lethal cross producing t^{w5} homozygotes were examined, a significant proportion also failed to label with anti-F9 serum. t^{w5} is a lethal acting at 6 days of development and belongs to a different complementation group from t^{w32} . As pointed out by Kemler *et al.*, since F9 is not expressed by two different *t* haplotypes it cannot at this time be considered the product of any particular wild-type allele of *t*. However it is clear that F9 expression is affected by a region belonging to or closely linked to the *T/t* locus.

The other major approach to serological aspects of the *T* locus has been the production of antisera against $+/t^x$ sperm and absorption with wild-type sperm to remove sperm autoantibodies. *T/t* locus mutations specify serologically detectable antigens on sperm, codominantly expressed, they are not present on other adult cells so far tested (Artzt & Bennett *Nature* **256**, 545; 1975; Yanagisawa *et al. Immunogenetics* **1**, 57; 1974). Serological analysis is difficult because the antisera are generally weak in cytotoxic reactions with sperm. Cross absorptions of anti- t^x sera with different *t* haplotype sperm have shown that these antisera detect multiple specificities, some of which may be unique for a given *t* haplotype. However it cannot be excluded that anti- t^x sera detect, in addition to a genuine t^x antigen, some other unknown antigen specified by a gene linked to *T/t*.

The experiments of Kemler *et al.* have shown that the *t* antigens defined on sperm are indeed expressed on embryos carrying the appropriate *t* haplotype. A sizeable fraction of embryos from crosses yielding $+/t^x$ heterozygotes are labelled by antisera against t^{w32} , t^{w5} , t^{w10} and t^{w18} haplotypes. The important point is that each of these *t* haplotype associated antigens is expressed at the morula stage. Thus it seems that although there seem to be *t*-stage specific lesions in development, there is no temporal correlation in the expression of the t^x antigens. There is, presumably no sequential switching on of genes coding for surface products which carry these antigens and which have been hypothesised to control important developmental events.

The evidence for the developmental

stage specificity of the *t* lethals has been largely derived from morphological studies of developing homozygous embryos *in utero*. The general interpretation has been that the *t* alleles affect specific cell types within the embryo. Recent work by Wudl and Sherman (*Cell* 9, 523; 1976) has suggested that some *t* mutations are general cell lethals and do not affect a single cell type. When individual t^{w5}/t^{w5} embryos are cultivated *in vitro*, all the different cellular derivatives die; control explants of normal embryos survive favourably in these conditions. Wudl and Sherman also claim that the t^6 mutation may be a simple cell lethal, although Erickson and Pedersen (*J. exp. Zool.* 193, 377; 1975) have obtained different results. Later acting t^{w18} mutations exhibit more specific cell lethality since homozygous embryos will develop in ectopic sites, are histologically anaplastic, but devoid of mesodermal derivatives (Artzt & Bennett *J. natn. Cancer Inst.* 48, 141; 1972). Thus it is possible that there are two classes of *t* mutations, those which are lethal for all embryonic cell types and those which have cells that can escape the homozygous condition.

Whether or not the molecules carrying the *t* antigens are involved in cellular interactions in development remains an open question. The complexity of the cellular specificity of *t* mutations, the *T/t* locus genetics and the antisera make the task of answering it difficult. The isolation in culture of cell lines carrying particular *t* haplotypes will avoid the problems associated with sperm cytotoxicity and eventually allow biochemical characterisation of these antigens as with the F9 (Jacob *Immun. Rev.* 33, 3; 1977). The identification of another antigen, PCC4, expressed by multipotent embryonal carcinoma and spermatozoa, but only on the inner cell mass of the newly implanted blastocyst (Gachelin *et al. Devl Biol.* 57, 199; 1977) lends support to the view that surface changes are co-incident with important determinative events in the embryos. □

Food lost to pests

from Robert M. May

FOR crops of grain in mediaeval times, a rough rule of thumb said that of every three grains, one was lost to pests or in storage, one was for next year's seed, leaving one to eat. The yield from modern crops is much greater, and the fraction to be set aside for seed is negligible, but the fraction lost has changed surprisingly little.

Drawing together information compiled by the US Department of Agriculture, Pimentel (*Bull. Ent. Soc. Am.*

Migration of a spreading axis

from Peter J. Smith

THAT part of the East Pacific rise which separates the Pacific plate to the west from the Nazca plate to the east between 0°S and 34°S is the most rapidly spreading section of the world oceanic ridge system. Some of it is also among the most complicated insofar as there may be two very small crustal plates lying between 22°S and 26°S and between 32°S and the triple junction at 34°S, respectively (Herron *Nature phys. Sci.* 240, 35; 1972). Elsewhere, however, the spreading régime appears to be fairly simple, not least in the area around 31°S which has recently been investigated by Rea (*Earth planet. Sci. Lett.* 34, 78; 1977). Here the topographic axis of the rise is now continuous and almost linear. But as Rea has discovered, it has not always been so.

Evidence for past deviations comes chiefly from the magnetic anomalies in the region. The axial anomaly is straight as is that associated with the Matuyama-Gauss reversal at 2.41 Myr. But the anomalies associated with the Olduvai event at 1.73 Myr and the Brunhes-Matuyama reversal at 0.7 Myr are curved. Moreover, in the southern part of the survey area the Jaramillo event anomaly at 0.92 Myr is offset about 10 km right laterally. Thus although the spreading régime may be simple now and may have been simple more than 2 Myr or so ago, things were evidently more complicated between times.

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

Rea interprets these data in terms of local migrations of the spreading axis, following Blakely (*Geology* 3, 35; 1975) who invoked a similar phenomenon to explain spreading rate variations in the northeast Pacific. The axis of the East Pacific rise at 31°S was straight about 2.41 Myr ago but then gradually became more and more concave to the east until it broke in such a way that the southern section was offset to the west compared with the northern section. The break soon healed, however, leaving a continuous, but curved, axis which gradually became straight again. Throughout much of this period the spreading was also asymmetric (greater towards the east) and the total spreading rate varied.

The details of these axial migrations are, of course, specific to the area under investigation; but the underlying processes are more general. It appears to be significant, for example, that when the axial offset occurred, the result was not to move the axis further in the direction of curvature but to return it to a more central position, presumably closer to the underlying magma source. In fast spreading areas the relatively thin lithosphere cannot confine the spreading centre as easily as can the much thicker lithosphere in slow spreading regions, and so the axis tends to waver. When the axial curvature becomes too severe, however, it is relieved by offsetting which reduces the geographical deviation between the ridge axis above and the magma source below.

22, 20; 1976) has shown that in the US in 1974 the losses in agriculture to insects were 13%, to diseases 12%, and to weeds 8%. This sums to 33% of the annual crop lost to pests, broadly defined. Corresponding average annual losses to pests throughout the decade 1951–1960 were 13% to insects, 12% to diseases, and 9% to weeds, and throughout the decade 1942–1951, 7% to insects, 11% to diseases, and 14% to weeds: over this span of time, losses to insects and plant diseases increased, while losses from weeds decreased.

Although the overall fraction of US crops lost to insects has increased despite the application of insecticides, important reductions of insect losses have been made on some crops. Thus

Robert M. May is the Class of 1877 Professor of Zoology in the University of Princeton.

the fraction of the potato yield lost to insects has declined from around 22% in 1910–1935 to 16% in 1942–1951 and 14% in 1951–1960. In contrast, losses in the apple crop (mainly due to codlin moth and apple maggot) in the same time intervals have been 10%, 12% and 13%; this reflects both decline in cultural controls formerly practised in orchards and increased emphasis on cosmetic aspects of marketing. For corn, a major grain crop, insect losses increased from around 4% in 1942–1951 to 12% in 1951–1960: contributing factors appear to be the continuous cultivation of corn on the same land year after year (increasing susceptibility to rootworm), and the planting of corn types that are less resistant to insect depredations. In all, about 1.2 billion pounds of pesticides (insecticides, herbicides, fungicides), or about 6 pounds per person, are at present

used annually in the US. These pesticides are not distributed uniformly among all crops; the non-food crops of cotton and tobacco for example, account for 50% of all insecticides used in agriculture.

Of particular interest are the patterns of control with pesticides compared with the broad range of bioenvironmental controls, which include cultural manipulations (such as crop rotation), natural enemies, and genetic resistance. Pimentel's survey of the USDA data shows that for insect pests, bioenvironmental controls are used on about twice as many acres as insecticidal methods (9% compared with 5%). For plant pathogens, some form of bioenvironmental control is used on 90% of acreages compared with only 0.5% on which fungicides are used. Crop rotations have been, and continue to be, a major control method for many crop diseases; the absence of a crop for a year or so can cause the population of its pathogens in the soil to die out. For weeds, the estimate is that bioenvironmentally controlled acreage beats that treated with herbicides by 80% to 15%. The relation between the life-history strategy of a pest and the best way to control it is a fascinating one, and the subject of much contemporary research (see *Nature* 264, 211; 1976).

In the US, crop losses from birds and mammals are small (estimated around 1%), although they can be significant for particular crops, such as cherries. Post-harvest losses are, however, substantial. Such losses of stored food to insects, rodents, and microorganisms are estimated around 9%. Adding pre- and post-harvest losses, we see that in the US a total of about 39% (33% plus 6%) of the total food supply is lost to pests.

For the world as a whole, crop losses to pests have been estimated to be about 35% (Cramer, in a journal deserving full citation, *Pflanzenschutz-nachrichten* 20, 1; 1967). This includes destruction by insects, pathogens, weeds, mammals, and birds. The latter two categories of loss are more severe in the tropics and sub-tropics than in temperate regions, but the first three categories remain predominant. Pimentel observes that available evidence tends to suggest that the "green revolution" technology has intensified losses due to pests; recent strains that have been bred with more attention to natural resistance to insects and pathogens may help reverse this trend. Annual world use of pesticides runs around 4.1 billion pounds, or 1 lb per person, with 34% of the total accounted for by North America, 45% by Western and Eastern Europe, and 21% by the rest of the world.

A rough estimate of world post-harvest losses is 20% (Pimentel *et al. Science* 190, 754; 1975), ranging from 9% in the US to 40% and 50% in some of the developing nations in the tropics. Adding these to the pre-harvest losses, an estimate of 48% (35% plus 13%) is obtained for the total food loss. While it may be argued that some of these estimates are a bit on the high side, the fact remains that pest populations (broadly defined) are consuming or destroying something like one-third to one-half of the world's food supply. This is a loss we can afford less and less as time goes on. □

The Earth's core

from C. Hazard

A RECENT paper in *The Moon* (16, 41; 1976) by R. A. Lyttleton must be of considerable interest to geophysics. The hypothesis that the Earth's core consists mainly of molten iron that drained to the centre quite early on in the Earth's history is so familiar that few seem ever to question its validity. That the core is liquid is demonstrated by the absence of shear waves in it, and that it is metallic is suggested by the requirement of a conducting medium for the electric currents proposed to account for the magnetic field. That the material is iron was conjectured largely on the belief that iron is the only heavy element sufficiently abundant to explain the high density in the core, and implicit in this is the assumption that the core-mantle boundary represents an abrupt change in chemical composition.

However, there is the less well popularised alternative hypothesis that the Earth is fairly undifferentiated throughout and that in fact the core-mantle discontinuity is a change in phase from a non-metallic solid state to a denser metallic liquid state maintained by the great pressure and high temperature within the core. That is, it is a second kind of phase change involving a much greater density increase than the recognised so-called 20°-discontinuity, which occurs at a depth of 413 km where the change is to a different crystalline form.

When the widely held belief was that the Earth in its early stages was molten throughout, the iron core hypothesis seemed to follow as a natural consequence of the heaviest components settling to the centre, but now that cosmogony points to an origin as

a cold body accreting from planetesimals in the original gas-dust solar nebula the phase-change hypothesis has obvious attractions. However, since the late 1940s when proposed by Ramsey (see *Mon. Not. R. Astr. Soc.* 110, 325; 1950), it has found little favour with geophysicists and geologists, although to the best of my recollection it used to be discussed favourably by P. M. S. Blackett in his undergraduate lectures at Manchester in the early 1950s. In recent years the hypothesis has been revived, and its consequences worked out in great detail by R. A. Lyttleton.

According to Lyttleton's development (*Proc. R. Soc.* A275, 1; 1963; *Proc. R. Soc.* A287, 471; 1965) of Ramsey's hypothesis, the Earth formed at sufficiently low temperature to be solid throughout. Then radioactive heating gradually raised the temperature of the central regions to a critical value (of a few thousand degrees K), at which time the catastrophic collapse in a matter of minutes demonstrated mathematically by Ramsey's theory resulted in the sudden formation of a large metallic liquid core. From known seismic data, this core has been shown by Lyttleton to have had a radius just over 2,000 km. Under the influence of continuing radioactivity it has grown steadily to its present radius of 3,473 km, with an accompanying decrease in the overall radius of the Earth of slightly more than 0.1 mm per year, enough to diminish the overall radius by nearly 400 km in 4.5 eons!

It is known that hydrogen just about doubles its density and goes over to a metallic form when subjected to a pressure only about half that at the core-mantle boundary but there is no direct evidence that terrestrial material could also undergo such a transformation. However, in his recent paper in *The Moon*, Lyttleton has produced a persuasive argument in support of the phase-change theory by showing that the contraction required on this hypothesis can quite naturally remove the well-known inconsistency in the tidal theory of the evolution of the lunar orbit, first encountered by Jeffreys in the 1920s and which has remained unresolved ever since (Jeffreys *Mon. Not. R. Astr. Soc.* 80, 309; 1920; *The Earth* edns 2-6, Cambridge University Press, 1920). Jeffreys endeavoured to account for the apparent secular accelerations of the Moon and Sun solely in terms of the lunar and solar couples resulting from tidal friction, but found that the actual observed ratio, 2.9, of these accelerations was far lower than the tidal-theory value of about 7.0. It is this discrepancy that Lyttleton now shows can be removed by introducing terms making allowance for a secularly decreasing moment of inertia of the

C. Hazard is a member of staff at the Institute of Astronomy, University of Cambridge.

Earth, and that the required rate of increase is in remarkable agreement with the previously calculated rate of decrease of the Earth's radius (*Proc. R. Soc. op cit.*; 1965).

Small as the rate of decrease may seem, it yet gives a change in the Earth's angular velocity comparable to that produced by the tidal couples, but of opposite sign and therefore accelerative as required. Lyttleton shows that while a similar but much smaller acceleration could also be produced by separation of iron into the core, such draining down is generally regarded as having occurred largely, if not entirely, early in the Earth's history, and even under the most favourable assumptions as to the present rate, it could not nearly account for the observed discrepancy. It would seem that unless the adherents of the iron core hypothesis can produce a comparably satisfactory alternative explanation for this discrepancy emerging from the standard Jeffreys tidal theory, Lyttleton has struck a telling blow for the phase-change hypothesis for the nature of the core. □

Beaded bubbles

from Robert W. Cahn

AN infinite monocrystalline element containing a second element in solid solution, not liable to any phase transformation, tends to a spatially uniform distribution of the dissolved element, and diffusion will persist until that stable terminal state is attained. That cautious opening sentence incorporates several provisos: if the solid consists of distinct crystal grains or, being less than infinite, is bounded by free surfaces, complications ensue. In general, solutes tend to segregate either to or away from free surfaces or grain boundaries; improved methods of microanalysis have allowed a considerable body of experimental information to be assembled concerning this process of 'Gibbsian' segregation (J. H. Westbrook, in *Interfaces Conference, Melbourne 1969* (ed. Giffkins, R. C., 283 (Butterworth, 1969); E. D. Hondros, *ibid.*, page 77). Hondros's extensive experimental work has led him to the conclusion that solutes with a small equilibrium bulk solubility (usually associated with very different solvent and solute atomic sizes) are particularly apt to segregate to interfaces and surfaces, thereby relieving lattice strain in the solvent. As Westbrook points out,

surface segregation is not restricted to external surfaces; it can apply equally to internal pores, such as are found in imperfectly sintered powder compacts. Westbrook emphasises the role of the binding energy that holds vacancies and solute atoms together; if this is high, any excess vacancies diffusing to a pore must drag solute atoms along with them.

Materials scientists responsible for the design of nuclear fuel elements are currently much concerned with pores—'voids' is the preferred term—in neutron-irradiated metals and ceramics. During irradiation (especially of the metal used to encapsulate the fuel) interstitial atoms and vacancies are formed in equal numbers and diffuse away to 'sinks', predominantly dislocations and grain boundaries. These sinks attract interstitial atoms more effectively than vacancies and the surplus vacancies then assemble to form small voids, visible in the electron microscope. The voids cause swelling of the material, a disease which must be kept within strict bounds for safety reasons. The principal strategy for minimising swelling is to choose suitable base metals and to maximise the concentration of effective sinks for interstitials and vacancies alike. Now, another strategy has been identified.

Some years ago, P. R. Okamoto and H. Wiedersich of the Argonne National Laboratory (*J. nucl. Mater.* **53**, 336; 1974) observed indications that solute elements segregate to radiation-induced voids in a stainless steel. The evidence was indirect—micrographic features indicating pronounced lattice distortion alleged to accompany the segregation—and it was not possible to identify the segregating species. The investigators suggested that it was interstitial solute atoms generated by the irradiation which segregate and lead to the high lattice distortion observed in the electron microscope. Thus Hondros's view that segregation acts to reduce lattice strains is not always in accord with observation.

The whole matter has now been clarified by an elegant set of experiments published by K. Farrell, J. Bentley and D. N. Braski of Oak Ridge National Laboratory (*Scripta Met.* **11**, 243; 1977). They irradiated aluminium foils to very high neutron doses, enough to generate about 0.4 atomic % silicon by $^{27}\text{Al} \rightarrow (\text{n}, \gamma) \rightarrow ^{28}\text{Al} \rightarrow ^{28}\text{Si} + \text{e}$. When the foils were thinned and examined by scanning transmission electron microscopy (STEM), they were seen to contain not only ordinary voids and silicon precipitates, as expected, but also some anomalous voids sitting on the surface of the foil or extending beyond the foil edge. In fact, these voids, bounded by a dark margin, were inhabiting empty space. Very

detailed electron microprobe experiments using the ultrafine STEM beam proved that these free-standing voids were coated with shells (4–11 nm thick) of (possibly amorphous) silicon. The aluminium surrounding them had been dissolved away during the thinning process, leaving hollow silicon spheres standing clear of the foils. Silicon has a small solubility in aluminium, and meets Hondros's criterion for ready Gibbsian segregation.

Farrell, Bentley and Braski point out that this substantial Gibbsian segregation offers the hope of influencing the growth rate of voids, especially in their early stages. The segregated solute must alter the specific surface energy at the void walls and thus affect the efficacy of the void in attracting vacancies—and hence must alter the swelling rate too. If Okamoto and Wiedersich are right in presuming that radiogenic interstitial solutes are peculiarly prone to Gibbsian segregation, then it may be possible to design alloys with initial solutes which, after radiation-induced transmutation, segregate to the incipient voids and inhibit their further growth. That would be an elegant way of setting one consequence of irradiation to neutralise another. □

The plasmopause revisited

from Michael J. Rycroft

WITHIN the charged particle environment of the Earth, which encompasses the ionosphere and magnetosphere, the plasmopause is a physically significant boundary. The plasmopause marks the boundary between the cool, relatively dense plasma of terrestrial origin and the hot, tenuous plasma of both terrestrial and solar wind origin which resides in the outer magnetosphere. This demarcation feature is aligned along lines of force of the Earth's magnetic field; the feet of these field lines touch the Earth's surface at near 60° magnetic latitude. They extend out into space, crossing the equatorial plane at a geocentric radial distance of ~ 4 Earth radii. Such field lines are termed $L \sim 4$ field lines, using the parameter L introduced by McIlwain as a co-ordinate to order observations of the van Allen radiation belt electrons.

The existence of the plasmopause was discovered independently by two different scientists almost 20 years ago.

Michael J. Rycroft is a Lecturer in the Department of Physics at the University of Southampton and an Adjunct Professor in the Department of Physics at the University of Houston.

Using observations made at Seattle and Stanford, D. L. Carpenter, of Stanford University, California, was studying whistlers. Audio-frequency electromagnetic radiation from lightning discharges, which penetrates the lower ionosphere and is guided from one hemisphere to the other by ducts of enhanced plasma density, which are themselves field-aligned, suffers dispersion. The characteristic signature of the whistler in the frequency-time domain is thus explained. As in much of geophysics, the received signal is the result of integration of a weighted varying physical parameter over the propagation path, and has to be interpreted either by comparison with model computations or by inversion. Using the former method with multipath whistlers, Carpenter showed that the plasma density profile in the equatorial plane decreases smoothly out to $L \sim 4$. There the profile often exhibits a 'knee'; the plasma density decreases sharply, within a fraction of an Earth radius, by an order of magnitude or more, to only $\sim 10^7 \text{ m}^{-3}$. (Carpenter's knee is now termed the plasmopause). He also showed that the plasmopause persisted for several hours at least, and that its L -value was a maximum at dusk, at the bulge in the plasmopause.

K. I. Gringauz, of the Space Research Institute of the USSR Academy of Sciences, Moscow, was studying measurements made by "charged particle traps" carried on the Luna (Lunik) 1 and 2 rockets launched to the Moon. These can be interpreted to yield positive ion density values at intervals along the spacecraft trajectory. With coworkers, he found that the plasma density could suddenly decrease markedly on the outgoing part of the trajectory.

Thus these *in situ* observations confirm the 'remote sensing' whistler observations. The results obtained by one technique complement those obtained by the other. The power of one method (low cost and good temporal coverage for example) is offset by the serendipity of whistler occurrence and their time-consuming analysis. The power of the other method (unambiguous measurement at a point in space) is offset by the high cost of satellite experiments and the fact that many hours—or even days—pass between successive observations at neighbouring points on a particular L -value. In the intervening time, the plasma density may have changed—by an order of magnitude—due to dynamical processes.

A symposium on the Physics of the Plasmopause was held in Trieste in September 1974, under the aegis of the European Geophysical Society. In a special issue of the journal *Annales de Géophysique* (31, No. 1, 1975), both

invited review and contributed papers were published. The subjects covered ranged from peculiar features of the mid-latitude ionosphere associated with the plasmopause to energetic charged particle phenomena, and included treatments of geomagnetic pulsations observed at $L \sim 4$ and of some relevant theoretical problems.

The variations of the L -value of the plasmopause with changing geomagnetic activity (which follows changes of parameters of the solar wind) and at different local times have been investigated by several authors. Most recently N. C. Maynard and J. M. Grebowsky, of the NASA Goddard Space Flight Center, Maryland, have performed such a study based on observations taken aboard the Explorer 45 (or S³-A) satellite. It is curious and, in retrospect, unfortunate that this satellite did not carry an instrument to measure the plasma density directly. However, an instrument to measure the d.c. electric field responds, at low plasma densities, to potentials associated with the Debye sheath around the satellite. As the satellite moves in its 7 h 49 min orbit outwards in the equatorial plane, towards its apogee at a geocentric distance of 5.24 Earth radii, it crosses the plasmopause; there the instrument becomes saturated due to these sheath potentials. Using coincident ground-based whistler and Explorer 45 observations, M. G. Morgan and Maynard (*J. geophys. Res.* **81**, 3992; 1976) showed that saturation occurred at a plasma density of $6 \times 10^7 \text{ m}^{-3}$, to within a factor of two.

In a recent paper, 'The plasmopause revisited' (*J. geophys. Res.* **82**, 1591; 1977), Maynard and Grebowsky have studied statistically the plasmopause position. They have used observations taken over 15 months and covering 1972 completely. The local time coverage was from 2300 LT, through noon to 0800 LT. They found that the plasmopause bulge occurs at 2000 or 2100 LT at geomagnetically quiet times, but migrates towards 1800 LT as magnetic activity increases. During quiet periods, a secondary bulge near local noon is found, confirming results obtained recently by K. I. Gringauz and V. V. Bezrukhikh.

Observations of the variation of the plasmopause position with both local time and geomagnetic activity have been interpreted in recent years in terms of the electric field within the magnetosphere. This results from the superposition of the electric field representing the corotation of the inner magnetospheric plasma with the Earth, 'tied' by the geomagnetic field lines, and the electric field resulting from the flow of the solar wind past the magnetosphere which drives the convection of the outer magnetospheric plasma

from the night-side to the day-side in the equatorial plane. This latter field may be at least partially 'shielded' from the day-side of the Earth, for example by the shorting-out action of the highly conducting, sunlit ionosphere or by the build-up of space charge layers. The fact that the bulge moves towards dusk, with increasing magnetic activity, indicates that this shielding becomes less effective for the larger convection electric fields occurring then. The secondary bulge in the plasmopause near noon can be explained by the projection of the Sq dynamo electric field, in the ionosphere, along geomagnetic field lines into the magnetosphere.

Maynard and Grebowsky note that the plasmopause L -value is rather better ordered when plotted against Dst , the index of ring current intensity, than when plotted against K_p , the three-hourly, global (planetary) index of geomagnetic activity. This may be explained either by the plasmopause controlling the loss of ring current ions, by way of an ion-cyclotron resonance (loss-cone) instability, or by both the plasmopause and the energetic ring current ions responding together to changes of the convection electric field.

For the future, work remains to be done in the realm of plasma physics, to improve our understanding of the behaviour of the plasmopause as a boundary layer between cool and hot plasmas. The importance of the plasmopause in controlling wave-particle interactions, between whistlers and energetic electrons for example, or between ion-cyclotron waves and multifarious ions, is becoming recognised more and more. Now is the era when we can test the hypotheses that we have developed for the Earth's magnetosphere by applying them to exciting new observations of planetary magnetospheres. Whether or not we have to make reappraisals will remain to be seen. □

A hundred years ago

ON FRIDAY night a series of interesting experiments with the Jablochhoff electric light took place at the West India Docks, under the direction of M. Denayrouze. The apparatus used for the occasion consisted merely of an electro-magnetic machine worked by a small steam-engine, some insulated wires, and the electric candles, which are the invention of M. Jablochhoff, and composed, as we have already described, of two carbons placed side by side with a slip of insulating substance between them, which burns away with the carbon exactly in the same way as the wax of a wax candle is consumed with the wick.

From *Nature* **16**, 21 June, 152; 1877.

review article

The 'universal' dielectric response

A. K. Jonscher*

A review of dielectric data for a wide range of solids proves the existence of a remarkable 'universality' of frequency and time responses which is essentially incompatible with the multiplicity of currently accepted detailed interpretations. Certain unique features of the universal behaviour strongly suggest the dominant role of many-body interactions.

THE dielectric response of solids has been the subject of extensive investigations for the best part of a century. Existing data cover an enormous range of frequencies from 10^{-6} to 10^{16} Hz, neither limit being absolute. When it comes to theoretical interpretations, the properties of gases and liquids at microwave to optical frequencies are the subject of powerful modern approaches, while the technologically most important response of solids at sub-GHz frequencies remains the subject of piecemeal approaches tailored to individual materials and often based on highly arbitrary models and analytical techniques. In this paper I propose to review some of the available experimental evidence on the frequency, time and temperature dependence of the dielectric parameters for a wide range of solids and to establish the existence of a very general or 'universal' pattern of behaviour which should provide a framework for the classification of a wide range of dielectric phenomena. I will then try to demonstrate the inadequacy of the existing theoretical treatments and I shall propose a novel approach based on many-body interactions. I shall be concerned exclusively with the frequency range below 1 GHz where phonon and quantum phenomena are negligible.

Definitions

A vacuum capacitor with an electric field E between its metallic plates has an interfacial charge $Q_0 = \epsilon_0 E$, where $\epsilon_0 = 10^7/4\pi c^2 = 8.845 \times 10^{-12}$ F/m is the dielectric permittivity of free space and c is the speed of light. If the field varies with time, the charge Q_0 follows exactly, there is no 'inertia' in the vacuum response. If the capacitor is filled with a material medium—gaseous, liquid or solid—the induced charge is increased by the polarisation P of the medium

$$Q = Q_0 + P = \epsilon_0(1 + \chi)E = \epsilon E \quad (1)$$

where ϵ is the dielectric permittivity and χ the susceptibility of the medium.

Since no material medium can follow instantaneously the variations of the field, there is a delay between the polarisation and the field. It is convenient to define the time response to a step-function field, $E(t) = 0$ for $t < 0$, $E(t) = E_0$ for $t > 0$, of the current

$$i(t) = dP/dt = \epsilon_0 E_0 f(t) \quad (2)$$

where $f(t)$ is the dielectric response function. The 'vacuum response' is represented as a δ -function at $t = 0$, but it cannot be resolved experimentally. The response of polarisation to an arbitrarily time-varying signal $E(t)$ is given by the convolution integral

$$P(t) = \epsilon_0 \int_0^\infty f(\tau) E(t-\tau) d\tau \quad (3)$$

which indicates that the system retains 'memory' of past excitations. In a harmonically varying field with radian frequency $\omega = 2\pi f$, f being the circular frequency in Hz, Fourier transformation of equation (3) gives the frequency dependence of polarisation

$$P(\omega) = \epsilon_0 \chi(\omega) E(\omega) \quad (4)$$

where $P(\omega)$ and $E(\omega)$ are Fourier components of $P(t)$ and $E(t)$, respectively, and $\chi(\omega)$ is the complex Fourier transform of $f(t)$

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) = \int_0^\infty f(t) \exp(i\omega t) dt \quad (5)$$

where $i = (-1)^{1/2}$. The real component χ' gives the component of polarisation in phase with the field, the imaginary component, known as the dielectric loss gives the quadrature component. In a field of peak amplitude E_0 the energy lost per radian is $\epsilon_0 \chi''(\omega) E_0^2/2$, and the power lost is $\sigma(\omega) E_0^2/2$, and this defines the alternating current (a.c.) conductivity

$$\sigma(\omega) = \sigma_0 + \epsilon_0 \omega \chi''(\omega) \quad (6)$$

showing the relationship between a.c. conductivity and dielectric loss, with the direct current (d.c.) conductivity, σ_0 , being included. Equation (5) shows that $\chi'(\omega)$ and $\chi''(\omega)$ are inter-related and they are, in fact, Hilbert transforms¹ of one another, known in this context as the Kramers-Kronig relations which are valid in the most general conditions, subject only to the linearity of response. It follows that any one of the three functions $\chi'(\omega)$, $\chi''(\omega)$ and $f(t)$ fully determines the response of the system and the other two may be derived from it.

Mechanisms of polarisation

Of the several possible polarisation mechanisms, induced atomic, or electronic, and also ionic lattice polarisations, respond so rapidly, that they are effectively instantaneous below GHz frequencies, contributing a purely real value, ϵ_∞ , to the permittivity. Permanent molecular dipoles, ionic defects of dipolar type (vacancy-interstitial pairs) and also slowly mobile hopping charge carriers of electronic, polaronic or ionic nature produce much slower responses so that the permittivity of a medium containing some or all of these may be written in the form

$$\epsilon(\omega) = \epsilon_\infty + \epsilon_0 \sum_\alpha \chi_\alpha(\omega) \quad (7)$$

where the index α labels the various mechanisms and χ_α are complex in the sense of equation (5).

*Chelsea College, University of London, Pulton Place, London SW6.

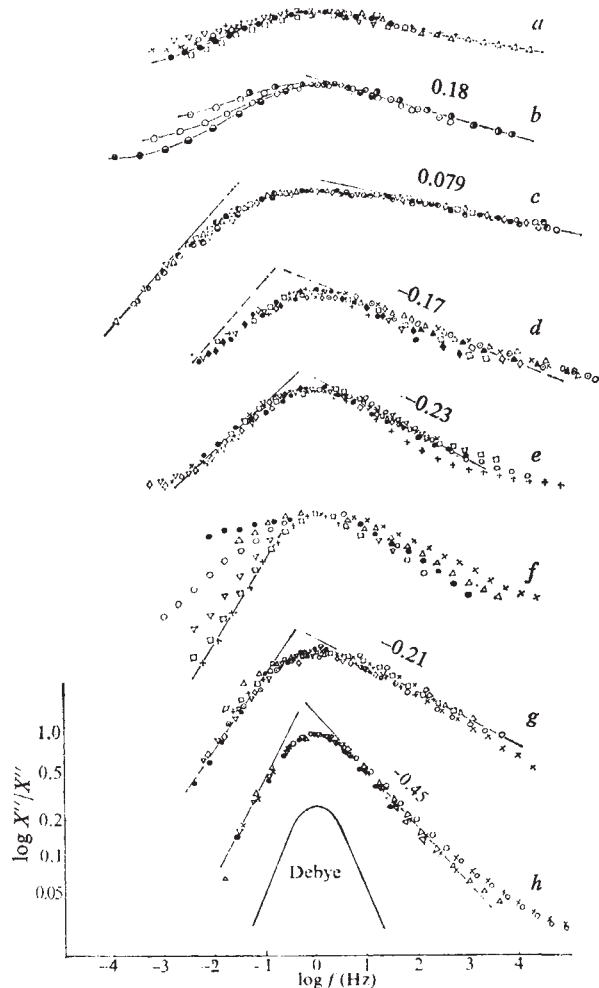


Fig. 1 A compilation of dielectric loss data for a range of polymeric materials. The points marked with different symbols correspond to data taken at different temperatures and transposed laterally to make the loss peaks coincide. The vertical scale is $\log \chi''/\chi''$ and the consecutive graphs are displaced vertically for clarity. The numbers indicate slopes of the straight line portions of the graphs. *a*, Low temperature data for polychloroprene; *b*, PCTFE 12% crystalline; *c*, polyethylene terephthalate; *d*, polymethyl methacrylate; *e*, high temperature data for polychloroprene; *f*, polyethyl methacrylate; *g*, polydian carbonate; *h*, polyvinyl acetate. The shape of a pure Debye peak is also included for comparison. (From ref. 5).

Dipolar responses

The classical case of dielectric response is that of a set of identical non-interacting dipoles free to rotate against some viscous resistance in a fluid-like medium—Debye's original model²—or 'jumping' by thermal excitation between two preferred orientations separated by a potential barrier—a situation more likely to be found in solids. The Debye susceptibility is given by the expression

$$\chi(\omega) \propto \frac{1}{1+i\omega\tau} = \frac{1}{1+\omega^2\tau^2} - i \frac{\omega\tau}{1+\omega^2\tau^2} \quad (8)$$

where the relaxation time τ is generally thermally activated and the loss peak frequency varies with temperature as

$$\omega_p = 1/\tau = \nu_0 \exp(-W/kT) \quad (9)$$

where ν_0 is some 'attempt-to-jump' frequency and W is an activation energy. The loss peak given by the second term in equation (8) is symmetric on a $\log \omega$ scale and has a width at half height of 1.144 decades.

Typical examples of dipolar behaviour may be found in many polar polymers³ in which the dipolar species may be well characterised both in type and in density. However, the dielectric loss in real polymers departs strongly from the Debye response and some examples are shown in Fig. 1 giving data corresponding to various temperatures 'normalised' by the customary displacement along the frequency axis to align the peaks^{4,5}. The Debye response is shown for comparison. The sharper α peaks are observed above the glass transition temperature, T_g and have approximately twice the width of Debye, the lower-temperature β peaks are hardly recognisable.

The main point I wish to emphasise here is that the post-peak response of all these materials may be characterised by the relation

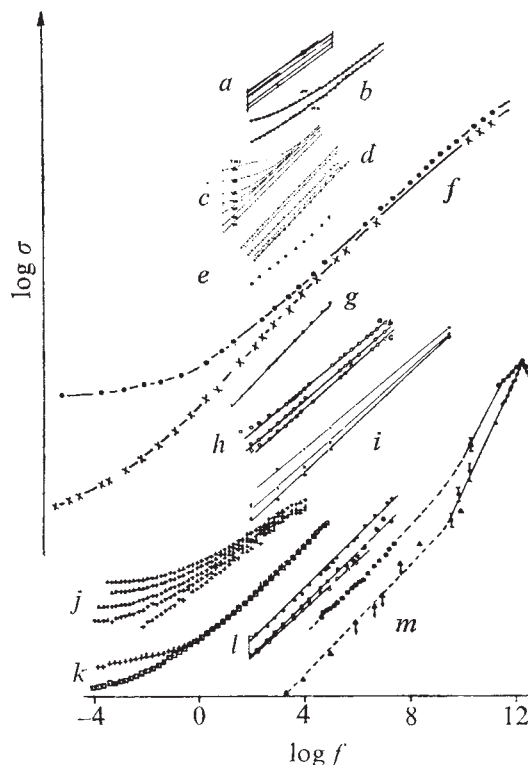


Fig. 2 The compilation of a.c. conductivity data for a range of materials arranged on a common $\log f$ basis (in Hertz) but displaced vertically along the $\log \sigma$ axis for clarity. Data sets denoted by one letter are on a common $\log \sigma$ scale. *a*, Single crystal silicon in the impurity hopping range by electrons at (bottom to top) 3.0, 4.2, 8.0 and 12.0 K. (ref. 13); *b*, single crystal β alumina at 77 and 87 K—a classic fast-ion conductor by Na^+ ion movement¹⁴; *c*, glow-discharge deposited amorphous silicon in the temperature range 84–295 K. (ref. 15); *d*, a range of chalcogenide glasses at 293 K, top to bottom: Sb_2S_3 , $\text{Sb}_{30}\text{As}_{10}\text{S}_{60}$, $\text{Sb}_{20}\text{As}_{20}\text{S}_{60}$, As_2S_3 , $\text{Sb}_{10}\text{As}_{30}\text{S}_{60}$ (ref. 16); *e*, single crystal anthracene at 294 K with 1 M saline solution as contact—two sets of measurements are shown (not resolvable on the scale of the present diagram)¹⁷; *f*, single crystal anthracene ($-\times-\times-$) and evaporated β carotene ($-\cdots-$) at 294 K (ref. 18). The two sets of data are on corresponding $\log \sigma$ scale, showing the close similarity of the results in the a.c. range, with significant departures in the d.c. levels. These results show the superposition of a very shallow peak at 10^6 Hz with an underlying trend $\sigma \propto \omega$; *g*, trinitrofluorone-polyvinyl carbazole (TNF PVK) (ref. 19); *h*, three glasses (a) 50 P_2O_5 -50 FeO , (b) 50 P_2O_5 -40 FeO -10 CaO , (c) 50 P_2O_5 -25 FeO -25 CaO : room temperature²⁰; *i*, 80V $_2\text{O}_5$ -20 P_2O_5 glass at three temperatures²¹; *j*, evaporated amorphous SiO_x (silicon monoxide) in the range of temperature (bottom to top) 211–297 K (ref. 22); *k*, stearic acid 9-layer film between Al and Au electrodes in the dark ($\square\square$) and in the presence of ultraviolet light ($+++$) (ref. 23); *l*, three amorphous samples (top to bottom) As_2Se_3 , Se and As_2S_3 . The measurements, taken at 300 K are believed to be those of the bulk material and not to be influenced by the electrodes²⁴; *m*, two samples of As_2Se_3 at room temperature²⁵ extending to far-infrared frequencies and showing a steeply rising ω^2 region above 10 GHz, characteristic of lattice vibrational processes.

$$\chi''(\omega) \propto \omega^{n-1} \quad \text{with } n < 1 \quad (10)$$

or by a superposition of two such functions with the higher-frequency component having values of n closer to unity. The exponents n are weakly temperature dependent, decreasing with increasing temperature. Many polymeric materials, polar as well as non-polar, show very flat losses over many decades of frequency, with superimposed very weak peaks, a behaviour consistent with $n \approx 1$ and not at all compatible with Debye-like responses.

A particularly interesting class of dipolar materials are clathrates⁶ in which 'guest' molecules are individually enclosed in cages of H₂O molecules having almost spherical symmetry. These materials show the same type of 'non-Debye' behaviour given by equation (10). There are also examples of solutions of polar molecules in non-polar solvents giving the same response not consistent with any obvious interpretation⁷.

Carrier polarisation

This category covers a very wide range of mechanisms and materials, the one common feature being that the charge carriers involved move by discontinuous hopping movements between localised sites—they may be electrons, polarons or various ions. Electrons or polarons normally hop between sites randomly distributed in space and in energy⁸ but it is almost impossible to distinguish between them experimentally. The d.c. conductivity is determined by the most difficult hops in percolation paths between the two electrodes, a.c. conductivity is thought to arise from more limited displacements, with two-centre hopping said to constitute the high-frequency limit. By contrast, ions move typically over much smaller, nearest-neighbour distances and it is particularly interesting to note, therefore, that neither the magnitude of the a.c. conductivity, its activation energy nor, in particular, its frequency dependence can be taken as reliable guides to the nature of the dominant carrier responsible for polarisation.

Electronic hopping is associated primarily with the class of amorphous and glassy semiconductors⁸⁻¹² which have received a good deal of experimental and theoretical attention in the last fifteen years or so. Figure 2 shows a selection of conductivity data for several of these materials¹³⁻²⁵, plotted on a common scale of $\log f$ and displaced vertically along the $\log \sigma(\omega)$ scale for clarity. The same figure also shows some other materials not necessarily associated with glassy electronic semiconductors, notably β alumina, the classic ionic conductor, as well as anthracene and β carotene.

The frequency dependence of the electrical conductivity of all these very different materials may be represented by the expression

$$\sigma(\omega) \propto \omega^n \quad n \leq 1 \quad (11)$$

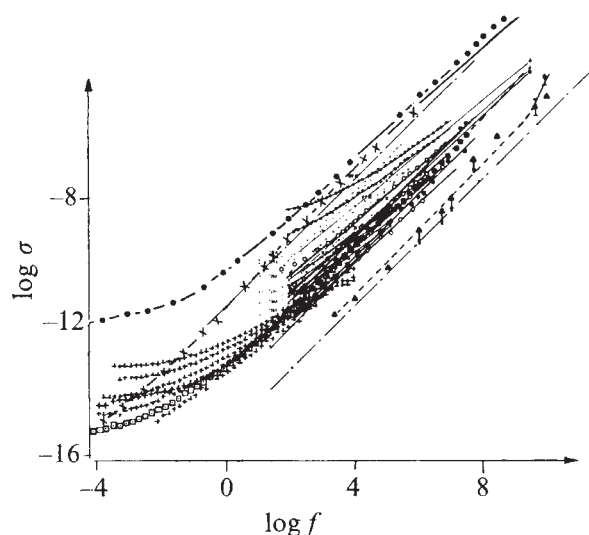


Fig. 3 The compilation of the data for the a.c. conductivity of Fig. 2 on a common scale of $\log \sigma$ ($\Omega \text{ cm}$)⁻¹ showing the relatively narrow range of a.c. conductivity for a very wide range of materials. The upper and lower sloping chain-dotted lines correspond, respectively, to dielectric loss ($\chi'' = 10$ and 10^{-3} , independent of frequency).

which is fully equivalent to (10). Most of the samples show no sign of d.c. conductivity or even of any deviation from a single value of the exponent n , while some show a smaller value of n at lower frequencies— b , c and k —yet others give a d.c. or near-d.c. dependence— j and k . The temperature dependence of the a.c. conductivity is generally much weaker than that of the corresponding σ_0 , the exponent n showing the same trend as in Fig. 1. Both β carotene and anthracene reveal a shallow loss peak superimposed on the general trend and centred on 1 MHz. The characteristic feature of all the other data is the absence of any evidence of loss peaks which would correspond to slopes significantly in excess of unity on the low-frequency side.

The numerical values of the exponents n for the different materials are listed in Table 1. The one conclusion that may be drawn is that values in the range $0.5 < n < 0.9$ are closely associated with proven carrier transport: hopping electrons in crystalline and amorphous silicon, SiO_x and stearic acid, the response of which is shown in more detail in Fig. 4. In this case it has been established that the values of n close to unity are to be associated with the 'lattice' response²⁶. This distinction between 'lattice' and 'carrier' responses corresponding, respectively, to 'intrinsic', that is irreducible, and

Table 1 Numerical values of the exponent n for different materials in Fig. 2

Diagram	Material	Temperature range (K)	Exponent n	Remarks
<i>a</i>	Single X-tal Si	3.0–12.0	0.79–0.74	
<i>b</i>	Single X-tal β alumina	77–87	0.75–0.70	high frequency
<i>c</i>	a Si	84–255	~ 0.5	low frequency
<i>d</i>	Sb _x As _{2-x} S ₃	293	0.96–0.90	
<i>l</i>	As ₂ Se ₃	300	1.0	
	Se	300	0.95	
	As ₂ S ₃	300	0.92	
<i>m</i>	As ₂ Se ₃	300	0.92	
<i>e</i>	Single X-tal anthracene	294	0.85	
<i>f</i>	Single X-tal anthracene	294	~ 0.95	shallow peak
	β carotene	294	~ 0.90	shallow peak
<i>g</i>	TNF–PVK		~ 1.0	
<i>h</i>	P ₂ O ₅ –FeO–CaO glass	300	0.85–0.89	
<i>i</i>	V ₂ O ₅ –P ₂ O ₅			
<i>j</i>	SiO _x	211–297	0.6–0.7	
<i>k</i>	Stearic Acid	300	0.95	high frequency
			0.6–0.7	low frequency

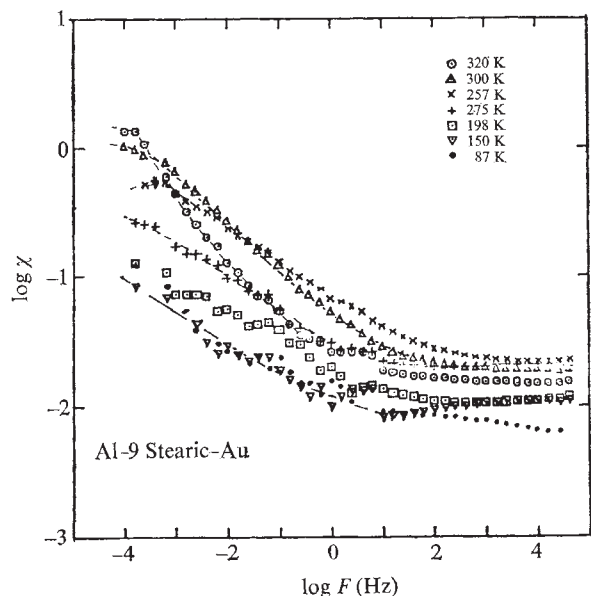


Fig. 4 The frequency dependence of the dielectric loss of a 9-layer structure of stearic acid between an aluminium base electrode and a gold top electrode, for a range of temperatures. From ref. 26.

'extrinsic' processes due to some impurities or injected carriers, may prove to be a useful diagnostic tool in the assessment of dielectric materials²⁷.

The expanded data of Fig. 2 are replotted again in Fig. 3 but this time on a common scale of $\log \sigma$, in order to bring out the very narrow range of values of $\sigma(\omega)$ at any particular frequency. The two sloping chain-dotted lines correspond to $\chi'' = 10$ and 10^{-3} independent of frequency, that is they cover four powers of ten and virtually all the data fall in this range, while a large majority actually fit into two decades, between 1 and 10^{-2} . This narrow range of the observed values of the loss was pointed out by me some time ago²², when I speculated on the possible role of contacts, as I found it difficult to believe that all these diverse materials should show such similar bulk properties. However, I do not think that there can be much doubt about the fact that we are dealing with genuine bulk properties and this is further confirmed by the very similar range of absolute values of dielectric loss in many polymeric solids.

Other dielectric materials

Many more examples may be quoted of dielectric behaviour following the general trend given by equations (10) and (11), with either a loss peak or a trend towards d.c. at low frequencies and a few of these will be mentioned here: sputtered films of lead-zinc-niobate and other materials^{29,30}; electrolytic capacitors of Al or Ta on plain foil, deeply etched foil or sintered porous bodies, thus proving that the geometrical configuration of the films has no significant influence on the response (F. Meca, personal communication); aqueous solutions of alkali halides, for example LiCl (ref. 31); dry sand follows equation (11) over seven decades with $n = 0.84$ (ref. 32); a wide range of composite dielectric materials such as rubbers, ceramics, sintered alumina, insulating glasses^{33,34}, and so on.

The same 'flat' frequency dependence of loss is found in some ferro-electric materials such as lead-zirconate-titanate and similar ceramics and likewise triglycene sulphate (TGS). In TGS the results are particularly striking since the Curie temperature, T_c , is at 50 °C and both χ'' and χ' show a singularity at T_c as functions of T , both being fairly flat in frequency at all temperatures, while the ratio χ''/χ' remains almost constant across the ferro-electric-para-electric transition (D.C. Dube, personal communication).

Very thin films also sometimes display the response characterised by equation (10). It is found down to monomolecular layers of, for example, stearic acid²⁶. Silicon P-N junctions form capacitors in one of the most perfect dielectrics known and it is therefore particularly interesting that their loss shows very shallow peaks resembling the flatter examples of dipolar losses in Fig. 1 (ref. 35). Interesting dielectric properties are shown by the solid electrolytes, defect solids in which ions show very high mobilities owing to the fact that there is no need to create the vacancies normally required for ionic conduction³⁶⁻³⁸. Many of these materials show negligible electronic conduction. Their electrical properties are commonly measured as complex impedance over a range of frequencies and the complex impedance diagrams are circular arcs with centres depressed below the real axis. I have pointed out that this is a direct consequence of a frequency-dependent conductivity of the type given by equation (11) and detailed studies confirm that this behaviour is found in a wide range of materials, single crystalline such as the β alumina in Fig. 2, polycrystalline and glassy (J. M. Reau, personal communication).

Time-domain response

Subject to the linearity of response, for example absence of charge injection⁴⁰, the response function $f(t)$, equations (2) and (5), corresponding to the 'non-Debye' response given by equation (10) is

$$f(t) \propto t^{-n} \quad (12)$$

and most dielectric materials show this form of current response to step-function excitation which is known as the Curie-von Schweidler law⁴¹⁻⁴⁴. Where a loss peak is present, as in Fig. 1, the response becomes⁴⁵

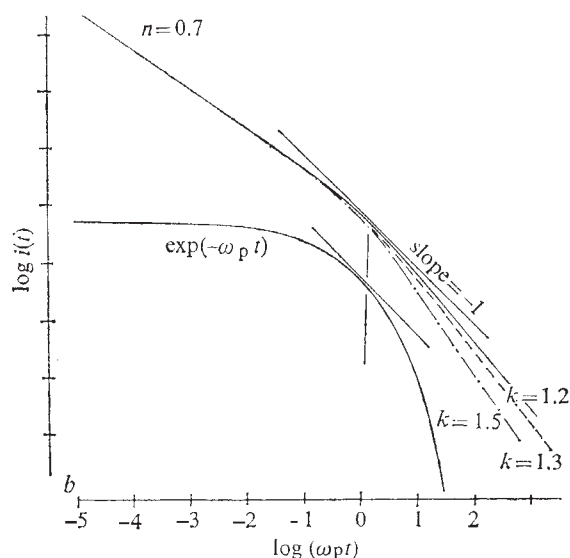
$$f(t) \propto [(\omega_p t)^{1+m} + (\omega_p t)^n]^{-1} \quad (13)$$

where $m < 1$, and this is represented in Fig. 5. Physically this corresponds to two consecutive processes, the Curie-von Schweidler law with the negative exponent $n < 1$ for times $t < 1/\omega_p$, and a similar law but with the negative exponent $1+m$ in excess of unity at times $t > 1/\omega_p$.

The 'universal' law of dielectric response

The dielectric response of almost all solid dielectrics—and of some liquid ones as well—may be represented by either of

Fig. 5 Current response under step-function excitation according to equation (13), with $n = 0.7$ and $k = 1+m = 1.2, 1.3$ and 1.5 . The slope becomes equal to -1 at $\omega_p t = 1$. The Debye response $\exp(-\omega_p t)$ is also shown for comparison. From ref. 45.



the two diagrams of Fig. 6; *a* represents the classical dipolar response modified by the experimentally observed broadening, while Fig. 6*b* represents the 'carrier' dominated low-frequency response. The common feature is the extended range of frequencies over which the loss obeys the law given by equation (10), which has the unique property that its Kramers–Kronig transform has the same functional form but for a constant factor, so that the real part of susceptibility is the same function of frequency and the ratio

$$\chi''(\omega)/\chi'(\omega) = \cot(n\pi/2) = \text{const.} \quad (14)$$

independent of frequency. This is in complete contrast with the Debye behaviour for which this ratio is equal to $\omega\tau$.

I define this as the 'universal' dielectric response and its range of validity extends from ω_p , in those cases where it is observable, to the onset of phonon and quantum phenomena at around 1–10 GHz. In many materials we have an unbroken range of six to ten decades of frequency, while in others it is easy to recognise the superposition of two mechanisms of this type but with different values of n .

As well as the range of frequencies, what is most remarkable is the range of material for which this universal law is applicable:

All structural forms: single crystal, polycrystalline, amorphous and glassy, aggregates; **all types of chemical bonds:** covalent, ionic, molecular; **all possible types of polarising species:** dipoles, hopping electrons, ions; **a wide range of geometrical configurations:** 'bulk' samples, thin films down to molecular dimensions, intricate geometries.

These facts have been known, albeit perhaps not in their full extent, for many years, some going back seventy years and

many attempts have been made to interpret this behaviour in terms of some model or other, according to the particular class of materials under consideration. A more detailed critique of this will be found elsewhere (A.K.J., to be published) and I have to limit the discussion to bare minimum here. The dipolar responses are frequently interpreted in terms of distributions of Debye-like relaxation processes, although it is difficult to see how the required range of τ , covering eight to ten decades of time can be justified in any particular system. Hopping electrons and polarons have been the subject of very extensive treatments^{46–55}, in terms of percolation theories and jump probabilities between states randomly distributed in space and in energy. While these treatments are very plausible and mathematically much more refined than many other dielectric theories, it is difficult to believe that the range of materials shown in Fig. 3 should exhibit such closely similar absolute values, given the wide range of physical hopping parameters likely to be involved. Moreover, the applicability of the universal law down to the thinnest samples presents some difficulties in this approach, where consecutive hops are needed to give the observed characteristics.

Ionic conductors are being interpreted in terms of some distributions of 'relaxation times' which are not specified and therefore are difficult to criticise. Alternatively, it is postulated that the bulk behaves in a frequency-independent manner, that is as an ideal dielectric in parallel with an ideal conductance, while the entire frequency dependence derives from limitation of ion supply across interfacial barriers^{56,57} whose transmission coefficients have to be postulated according to need. However, these coefficients are not separately measurable so they remain conjectural and, in any case, it is difficult to explain by this means values of n in the range 0.5–0.8 typically found in many ionic conductors.

Attempts have been made to move away from Debye philosophy by introducing diffusion-limited relaxations^{58–60}, but apart from a certain lack of physical clarity regarding the mechanism, it is only possible to explain by this means values of n close to 0.5. Diffusion is also at the base of the Warburg impedance mechanism⁶¹ commonly used in liquid electrolyte problems and often invoked in solid electrolytes, although its applicability here is suspect since it requires the presence of two mobile ionic species with opposite signs of charge.

Many analytical attempts have been introduced to explain the time-domain Curie–von Schweidler law in terms of correlation functions^{62–64} but again one is left with an essential arbitrariness at some stage in the choice of the functions required to give agreement with experiment.

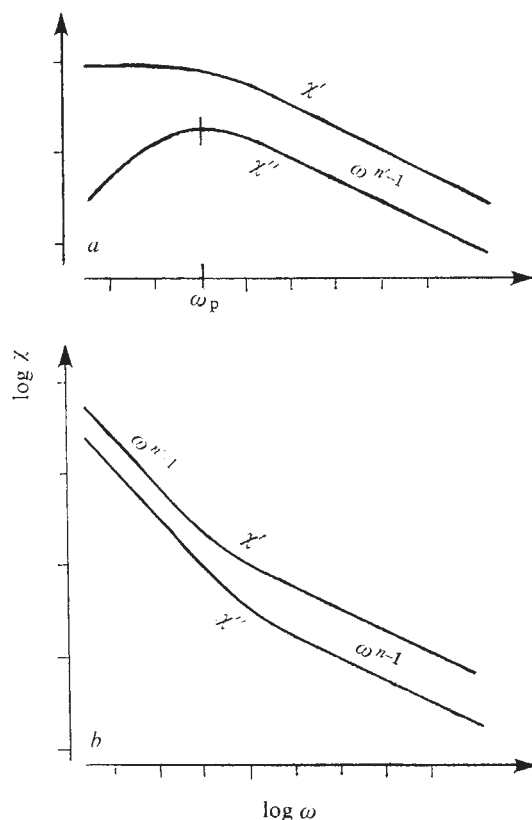
Many-body interactions

I suggest that, faced with the sheer universality of the observed behaviour, in terms not only of a common frequency dependence but, not least, of a narrow range of absolute values of loss in very diverse materials, one is left with the overriding impression of an essential insufficiency of all these different interpretations and the need for an entirely fresh approach, based on a principle of sufficient inherent generality to be capable of embracing all the materials under consideration.

What is even more remarkable is that the universality does not stop with dielectric responses—it is well established in the case of mechanical moduli of disordered systems, both polymeric⁶⁵ and metallic. Here I refer not to the well known fact that dielectric and mechanical loss peaks follow similar trends⁶⁶, but to the much more significant, in the present context, relaxation law beyond the peaks which is of the form (10), even though the exponent n is not the same in both cases. There is also a well established equivalent of the Curie–von Schweidler law in mechanical relaxation.

There is even some evidence, although much less well documented, that magnetic losses in many ferrites follow an almost frequency-independent trend at low frequencies⁶⁷, thereby tentatively extending the observed universality of the dynamic relaxation to the third fundamental stress–strain relationship.

Fig. 6 Schematic representation of the two types of dielectric response in the frequency domain, plotted as the logarithm of the real and imaginary components of the susceptibility against the logarithm of frequency. Both show the 'universal' behaviour with ω^{n-1} dependence at the higher frequencies. Diagram (*a*) corresponds to a dipole-dominated response, with a loss peak at the ω_p , diagram (*b*) gives case carrier-dominated low-frequency response with a different value of the exponent $n' < n$. The scale markers signify units on the logarithmic scales.



I have suggested that the universally observed constancy of the ratio (14) implies the physically elementary fact that⁵

$$\frac{\text{energy lost per radian}}{\text{energy stored}} = \cot(\pi/2) = \text{const.} \quad (15)$$

independently of frequency and that this should be taken as the universal criterion of dielectric, mechanical and, possibly even magnetic relaxations in solids.

In the case of dielectrics, I have noted that the one general property of all systems under consideration is that the polarisation is due to discontinuously hopping electrons or ions, or to likewise discontinuously jumping dipoles and I have introduced the simple concept of screening of charges and dipoles. This screening is the result of many-body interactions and has the simple and universal property that it adjusts 'slowly' to the 'fast', that is effectively discontinuous, jumps of individual particles or dipoles. I have suggested that this 'dual' time scale leads to the property (15) and, therefore, inescapably through the Kramers-Kronig relations, to the universal frequency dependence (10).

The introduction of many-body formalism into the interpretation of the universal dielectric response of solids does find a striking parallel in the theory of X-ray singularities in metals⁶⁸ and also in the problem of infrared divergence⁶⁹, both leading to theoretical expressions for time-dependent responses resulting from many-body interactions which are identical with the Curie-von Schweidler law. While the time scales in question are separated by many orders of magnitude, the similarity of the resulting power-law dependence on time invites a detailed examination of the validity of this approach. (This study is currently being carried out in collaboration with K. L. Ngai of the Naval Research Laboratory, Washington DC.)

An attempt to explain the conductivity law (11) in terms of many-body interactions was made by Pollak and Pike⁷⁰, who associated it with the low-temperature specific heat anomaly in glasses. An even earlier suggestion to this effect was made by Garton⁷¹ but seems to have been largely forgotten. These are clear pointers in the direction of many-body interactions being an acceptable explanation of the universal dielectric response, but a great deal of work requires to be done before the situation could be regarded as clarified. I am suggesting that the dielectric response provides us with an 'analogue solution' of a many-body interaction and this may prove valuable in the theoretical studies. In addition, I believe that the physical significance of the energy criterion (15) in the frequency domain should not be underestimated—it may give us a vital insight into the processes in question, mechanical no less than dielectric. My screened-hopping model is based on this criterion, but there may exist other possible ones.

The loss peak and other low-frequency processes

To the extent to which we may accept the many-body approach, through the energy criterion, as explaining the universal response in the frequency domain, equation (10), in the range in which it is valid, we need an interpretation of the loss peaks which are found in dipolar systems. I suggest that their interpretation in the accepted 'distribution' argument is not very satisfactory, not least in view of the fact that the pre-exponential factor v_0 in equation (9) is found to cover the prodigious but physically meaningless range 10^3 to 10^{30} Hz, for both mechanical and dielectric cases (C. Lacabanne, personal communication), where 10^{13} would have been expected as a 'lattice' frequency.

I have suggested that a very simple explanation of loss peaks in the context of our many-body approach may be found in the time-domain picture of Fig. 5 (ref. 45). The loss peak arises from the transition in the time-domain response after a step function excitation, from a 'primary' relaxation process with $n < 1$, to a 'secondary' process with a larger exponent which completes the adjustment to the steady state. The primary

process is the Curie-von Schweidler law, corresponding to the energy criterion in the frequency domain. The secondary process is not at present understood, although its physical necessity in the case of dipolar polarisation follows from the requirement to have a finite total charge in the system. There is, however, nothing inherently implausible in a concept of a succession of two different processes in a many-body relaxation. The important point is that the loss peak is the resultant of this transition from one process to another and that the loss peak frequency is determined solely by the time of transition. That this time should be activated with a well defined energy, as is normally observed, seems perfectly understandable—it must be related to some thermal processes, but there is no need to associate the pre-exponential factor v_0 with any particular 'lattice' frequency, as is the case in the Debye philosophy.

The apparent absence of loss peaks in carrier-dominated situations, as well as the presence in some cases of strong low-frequency dispersions obeying an approximately $1/\omega$ law for both $\chi'(\omega)$ and $\chi''(\omega)$ presents an interesting problem but lies beyond the scope of the present treatment.

Conclusions

Experimental evidence leads to the conclusion that there exists for a wide range of solids a universality of dielectric behaviour demanding a much more unified interpretation than the hitherto accepted specialised approaches which are somewhat arbitrary and which are tailored to the various separate classes of materials. Moreover, the universality extends beyond the dielectric properties—it is well established in the case of dynamic mechanical response of glassy solids and there is tentative evidence for its presence in magnetic response.

I suggest that the unified approach should be based on the indisputable common property of all condensed matter—the many-body interactions between its constituent parts.

The otherwise somewhat arbitrary functional form of the universal response has the unique property in the frequency domain that the ratio of energy lost per cycle to energy stored is independent of frequency and I am proposing to make this the physical criterion of all dielectric responses. The corresponding time dependences are identical with those found theoretically for a number of many-body interactions, such as the X-ray singularity and the infrared anomaly.

In the dielectric case this criterion is satisfied by mutual screening of hopping charges or dipoles and is based on the relatively slow adjustment of screening in comparison with the practically instantaneous hopping transitions of individual charges or dipoles. A new interpretation is proposed in the same framework of the peaks of loss observed in dipolar systems.

This work was carried out under financial assistance from the Science Research Council and from Chelsea College. I am grateful to many colleagues for helpful criticisms and to some for permission to quote unpublished information. I am particularly indebted to Dr. K. L. Ngai for his comments on the many-body interactions.

- ¹ Erdelyi, A. *Tables of Integral Transforms* 2, 249 (McGraw-Hill, New York, 1954).
- ² Debye, P. *Polar Molecules* (Dover, New York, 1945).
- ³ McCrum, N. G., Read, B. E. & Williams, G. *Anelastic and Dielectric Effects in Polymeric Solids* (Wiley, New York, 1967).
- ⁴ Jonscher, A. K. *Colloid and Polymer Sci.* 253, 231–250 (1975).
- ⁵ Jonscher, A. K. *Nature* 253, 717–719 (1975).
- ⁶ Gough, S. R., Hawkins, R. E., Morris, B. & Davidson, D. W. *J. phys. Chem.* 77, 2969 (1973).
- ⁷ Johari, G. P. & Smyth, C. P. *J. chem. Phys.* 56, 4411 (1972).
- ⁸ Mott, N. F. & Davis, E. A. *Electronic Processes in Non-Crystalline Solids* (Clarendon, London, 1971).
- ⁹ *Electronic and Structural Properties of Amorphous Semiconductors, Proceedings of the Scottish Universities Summer School in Physics 1972* (eds Le Comber, P. G. & Mort, J.) (Academic, New York, 1973).
- ¹⁰ *Amorphous and Liquid Semiconductors* (eds Stuke, J. & Brenig, W.) (Taylor and Francis, London, 1974).
- ¹¹ Jonscher, A. K. & Hill, R. M. *Physics of Thin Films* 8, 169–249 (1975).
- ¹² Böttger, H. & Bryksin, V. V. *Phys. Stat. sol.* (b) 78, 9 and 415 (1976).
- ¹³ Pollak, M. & Geballe, T. H. *Phys. Rev.* 122, 1745 (1961).
- ¹⁴ Grant, R. J., Hodge, I. M., Ingram, M. D. & West, A. R. *Nature* 266, 42 (1977).
- ¹⁵ Abkovitz, M., Le Comber, P. G. & Spear, W. E. *Commun. Phys.* 1, 175 (1976).
- ¹⁶ Roberts, G. C. & Polanco, J. I. *Solid State Comm.* 10, 709 (1972).
- ¹⁷ Abkovitz, M., Blossley, D. F. & Lakatos, A. I. *Phys. Rev. B* 12, 3400 (1975).
- ¹⁸ Hughes, D. & Pethig, R. in *Dielectric Materials, Measurements and Applications*, IEE Publication 129, 52 (IEE, London, 1975).

- 19 Abkovitz M., Lakatos, A. I. & Scher, H. *Phys. Rev. B* 9, 1813 (1974).
- 20 Murawski, L. & Gzowski, O. *Phys. Stat. sol. (a)* 24, K115 (1974).
- 21 Sayer, M., Mansingh, A., Reeves, J. M. & Rosenblatt, C. *J. appl. Phys.* 42, 2857 (1971).
- 22 Frost, M. S. & Jonscher, A. K. *Thin Solid Films* 29, 7-18 (1975).
- 23 Careem, M. A., Jonscher, A. K. & Taiedy, F. *Phil. Mag.* 35, No. 6 (1977).
- 24 Lakatos, A. I. & Abkovitz, M. *Phys. Rev. B* 3, 1791 (1971).
- 25 Strom, U. & Taylor, P. C. *Amorphous and Liquid Semiconductors* (eds Stuke, J. & Brenig, W.) 375 (Taylor and Francis London, 1974).
- 26 Careem, M. A. & Jonscher, A. K. *Phil. Mag.* 35, No. 6 (1977).
- 27 Jonscher, A. K. in *1972 Annual Report on Conference on Electrical Insulation and Dielectric Phenomena* 418 (Natl. Academy Sci., Washington DC, 1973).
- 28 Jonscher, A. K. *J. Phys. C* 6, L235-9 (1973).
- 29 Barrière A., Danto, Y. & Salardenne, J. *Thin Solid Films* 26, 273 (1975).
- 30 Danto, Y. thesis, Bordeaux Univ. (1975).
- 31 Angell, C. A., Bressel, R. D. & Gammell, P. J. *non-cryst. Solids* 7, 295 (1972).
- 32 Shahidi, M., Hasted, J. B. & Jonscher, A. K. *Nature* 258, 595-597 (1975).
- 33 Jonscher, A. K. *Thin Solid Films* 36, 1-20 (1976).
- 34 von Hippel, A. *Dielectric Materials and Applications* (Wiley, New York, 1954).
- 35 Barsony, I. & Jonscher, A. K. *Solid State Electronics* (in the press).
- 36 Kleitz, M. & Dupuy, J. (eds) *Electrode Processes in Solid State Ionics* (Dreider, Dordrecht, 1976).
- 37 Mahan, G. D. & Roth, W. L. (eds) *Superionic Conductors* (Plenum Press, New York, 1976).
- 38 Jonscher, A. K. & Reau, J-M. (in the press).
- 39 Jonscher, A. K. *Phys. Stat. sol. (a)* 32, 665 (1975).
- 40 Jonscher, A. K. & Careem, M. A. *Physics Letters* 55A 257-259 (1975).
- 41 von Schweidler, E. *Ann. d. Physik* 24, 711 (1907).
- 42 Vanderschueren, J. thesis, Liège Univ. (19).
- 43 Das Gupta, D. K. & Joyner, K. J. *Phys. D* 9, 829 and 2041 (1976).
- 44 Baird, M. E. *Rev. mod. Phys.* 40, 219 (1968).
- 45 Jonscher, A. K. *Nature* 256, 566-568 (1975).
- 46 Pollak, M. *Phys. Rev. A* 133, 564 (1964).
- 47 Pollak, M. *Phil. Mag.* 23, 519 (1971).
- 48 Jonscher, A. K. *J. non-cryst. Solids* 8-10, 293 (1972).
- 49 Scher, H. & Lax, M. *Phys. Rev. B* 7, 4491, 4502 (1973).
- 50 Butcher, P. N. & Morys, P. J. *Phys. C* 6, 2147 (1973).
- 51 Moore, E. J. *J. Phys. C* 7, 339 (1974).
- 52 Moore, E. J. *J. Phys. C* 7, 1840 (1974).
- 53 Halpern, V. *Physica* 79B 323, 336 (1975).
- 54 Austin, I. G. & Mott, N. F. *Advan. Phys.* 18, 41 (1969).
- 55 Davis, E. A. & Mott, N. F. *Phil. Mag.* 22, 903 (1970).
- 56 Macdonald J. Ross *J. chem. Phys.* 61, 3977 (1974).
- 57 Macdonald J. Ross *Electrode Processes in Solid State Ionics* p149 of ref. 36.
- 58 Glarum, S. H. *J. chem. Phys.* 33, 1371 (1960).
- 59 Glarum, S. H. *Molecular Phys.* 27, 1139 (1974).
- 60 Bordewijk, P. *Chem. Phys. Lett.* 32, 592 (1975).
- 61 Warburg, E. *Ann. d. Phys. Chemie.* 67, 493 (1899); *Ann. d. Phys. (Germany)* (4) 6, 125 (1901).
- 62 Williams, G. & Watts, D. C. *Trans. Faraday Soc.*, 66, 80 (1970); *ibid.* 67, 1323 (1971).
- 63 Wylie, G. in *Dielectric and Related Molecular Processes* 1, 21-64 (The Chemical Society, London, 1972).
- 64 Cook, M., Watts, D. C. & Williams, G. *Trans. Faraday Soc.* 66, 2503 (1970).
- 65 Ferry, J. D. *Viscoelastic Properties of Polymers*, 2nd edn (Wiley, New York, 1970).
- 66 McCall, D. W. in *Molecular Dynamics and Structure of Solids, NBS Special Publ. No. 301* (US Department of Commerce, 1969), 475-537.
- 67 Neel, L. J. *Phys. Radium* 11, 49 (1950).
- 68 Mahan, G. D. *Solid State Physics* 29 (eds Seitz, F. & Turnbull) 75 (Academic New York, 1974).
- 69 Hopfield, J. J. *Comm. Solid State Physics* 11, 40 (1969).
- 70 Pollak, M. & Pike, G. E. *Phys. Rev. Lett.* 28, 1449 (1972).
- 71 Garton, C. G. *Trans. Faraday Soc.* 42A, 56 (1946).
- 72 Berry, B. S. in *Metallic Glasses* (eds Gilman, J. J. & Leary, H. J.) (Am. Soc. Metals, Cleveland, Ohio, 1977).

articles

Turning points in Phanerozoic history

Martin A. Whyte

Department of Geology, University of Sheffield, Sheffield, UK

During the last 450 Myr there has been concomitant variation in the Earth's rotation rate, the polarity bias of the geomagnetic field, the amount of activity at ocean ridges, sea level and climate. At turning points when trends in the variables reverse themselves, climatic instabilities have disrupted biological ecosystems and led to mass extinctions.

ONE of the most interesting cooperative efforts within the earth sciences is that between palaeontologists and geophysicists in which skeletal growth rhythms recorded in fossils are used to elucidate the Earth's rotational history. It has been established that the rotation rate has fluctuated during the Phanerozoic. This synthesis suggests that subtle long term variations in other parameters can be correlated with changes in rotation rate.

Rotational history

The modification of skeletal growth by external environmental cycles can produce within a shell a detailed record of daily, fortnightly, monthly and annual events. The potential of such structures was first fully realised by Wells¹ who made counts on Middle Devonian corals which indicated that at that time, 390 Myr, there were 400 solar days in the year. As Wells^{1,2} appreciated the slowing down of the Earth's rate of rotation, which this result indicated, corroborated the geophysical prediction that the lunar tidal torque has a braking effect by which angular momentum is transferred from the Earth to the Moon. Soon afterwards Scrutton³ who also used Middle Devonian corals, made counts indicating that there had been 30.6 d in the synodic month and Runcorn⁴ showed how this pair of observations could be used to test for possible changes

in the Earth's moment of inertia. Runcorn's analysis, though probably unreliable due to the statistical uncertainty inherent in the original data^{5,6}, suggested that any changes in the Earth's moment of inertia must have been small and subsequent workers have generally accepted tidal friction as the operative mechanism in the rotation deceleration. However, as more palaeontological data became available it became evident^{7,8} that there had not been a uniform rate of decrease in spin velocity and a significant recent advance was made by Creer⁹ who recognised that there have been periods of earth history when the Earth's rate of rotation has accelerated (Fig. 1). During the last 500 Myr these periods of acceleration have alternated with periods of deceleration of spin velocity and there are turning points of two types, firstly spin maxima at transitions from acceleration to deceleration and secondly spin minima at transitions from deceleration to acceleration. Revised estimates indicate that spin maxima occurred 65 Myr (end Cretaceous) and 375 Myr (middle Upper Devonian) ago and that spin minima occurred 235 Myr (end Permian) and 445 Myr (late Ordovician) ago (Fig. 1). The periods of acceleration recognised by Creer⁹ cannot be easily explained by tidal friction alone and suggest that some additional mechanism or mechanisms must be operating. This additional component seems to be a fluctuating system superimposed on a general trend for deceleration of spin velocity which presumably represents the effects of tidal friction (Fig. 1).

Creer⁹ also recognised that the turning points were closely associated in time with periods of instability in the palaeomagnetic field (Fig. 1). While periods of rotational acceleration are characterised by a predominance of normal polarity and periods of rotational deceleration are characterised by a predominance of reversed polarity, turning points are associated with phases of mixed polarity which show no obvious polarity bias and which may also have

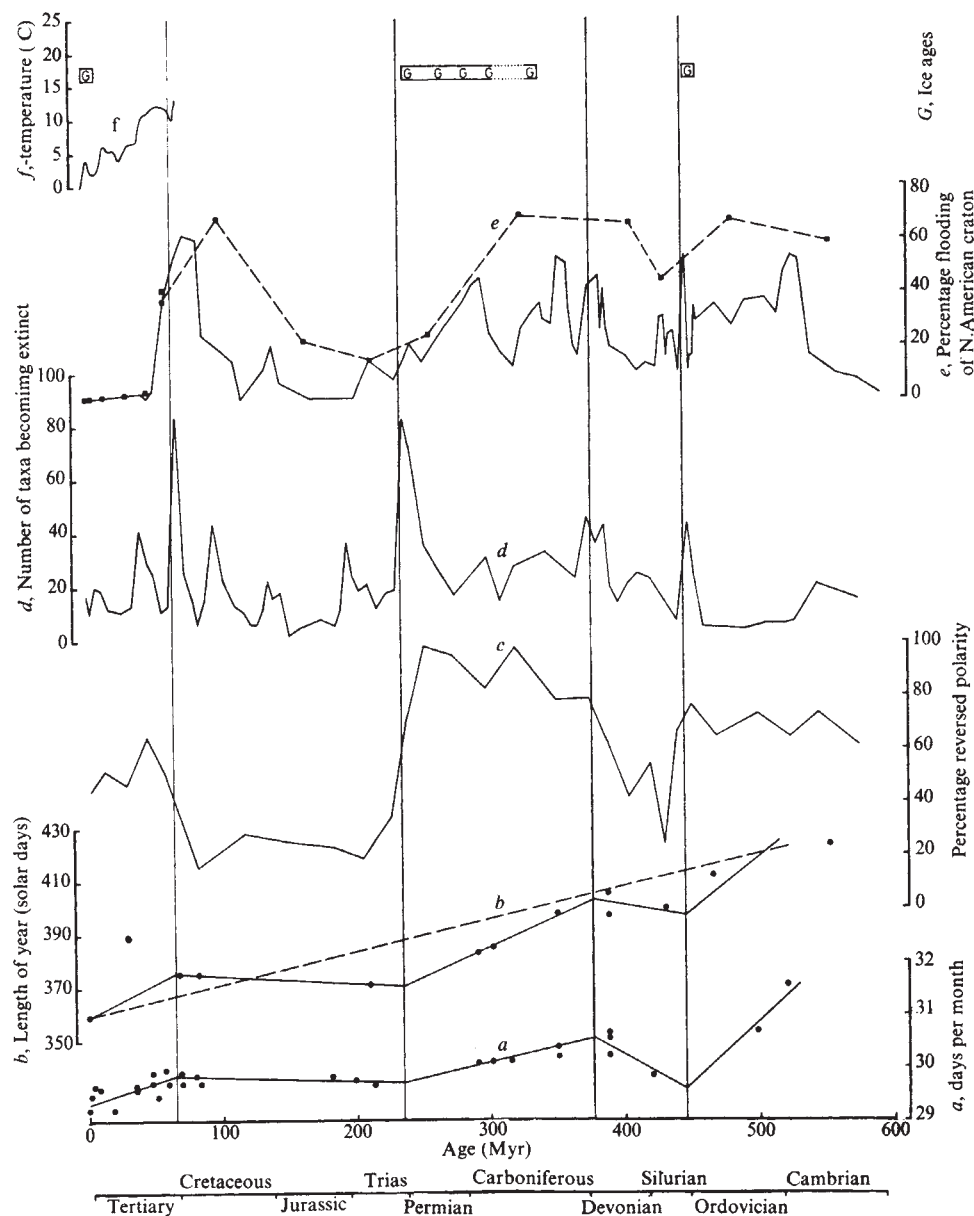


Fig. 1 Aspects of Phanerozoic history. *a*, Number of solar days per month (modified from Creer⁵ using palaeontological data⁷⁻¹³). *b*, Number of solar days per year (modified from Creer⁵; using palaeontological data 1, 2, 8, 14; the dashed line is extrapolated from the present rate of deceleration¹⁵). *c*, Percentage of reversed polarity (based on Creer⁵). *d*, Number of higher taxa, of all biological groups, becoming extinct (based on Cutbill and Funnell³⁸). *e*, Percentage flooding of the N. American craton (based on Wise⁵³; the dashed curve is a period-by-period smoothed curve). *f*, Temperature of high latitude marine waters (based on Savin *et al.*³⁹). *g*, Occurrence of ice ages (refs 43, 45-47).

had high reversal frequencies. A close link between the Earth's rotation and its magnetic field has long been recognised: there seems to have been a near alignment of rotational and dipole axes throughout time and it has been suggested that the influence of rotational forces on the fluid outer core may be vital to the generation of the magnetic field¹⁶. On the other hand the irregular decade fluctuations in the Earth's rate of rotation have been correlated with changes in the geomagnetic field and attributed to the transfer of angular momentum between the core and the mantle¹⁷. The core-mantle coupling is generally interpreted as being an electromagnetic effect though viscous forces and topographic features of the boundary may also be important¹⁸. Though extraterrestrial sources cannot be altogether discounted, the geologically detectable rotational and magnetic fluctuations are most probably due either to changes in the Earth's moment of inertia^{5,19} or to an internal redistribution of angular momentum¹⁹ and it is a great pity that the palaeontological data are not yet sufficiently precise to allow elimination of one or other of these possibilities using an extended form of Runcorn's analysis.

In the middle Carboniferous and again during the Late

Jurassic and early Cretaceous there were periods in which the palaeomagnetic field became moderately disturbed^{5,20}. However, unlike the conditions at turning points, the field retained an overall polarity bias and there was no associated switch in the direction of the predominant polarity. One might predict that these events were accompanied by slight inflections in the rate of change of the Earth's rotation but the palaeontological data are at present too scanty to allow this possibility to be tested. These episodes could perhaps be interpreted as aborted turning points and it is worth noting that they are followed by the most important Phanerozoic quiet intervals namely the Permian-Carboniferous (Kiaman) Reversed Interval and the Cretaceous (Mercanton) Normal Interval. The Cenozoic magnetic field has apparently always been relatively unstable but there seems to have been within the last 10 Myr a change from a predominance of reversed polarity^{5,20} (Fig. 1) and during the same period there has also been an increase in the frequency of reversals²¹. Astronomical estimates of the present rate of secular deceleration give a value lower than that which seems to have operated during the last 65 Myr (Fig. 1) and this, together with the magnetic evidence, might indicate that we are at or close

to another spin minimum. Including this putative turning point and the two aborted ones it seems that turning points are separated by a characteristic time interval with an average length of 74 Myr.

Mass extinctions

Several investigations²² of Pleistocene deposits have noted a significant correlation between magnetic reversals and extinctions of fossil species and these results are consistent with Uffen's hypothesis²³ that during reversals biological systems would be adversely affected by increased radiation doses. Simpson²⁴ extended this hypothesis to suggest a correlation between mass extinctions and periods of high reversal frequency and this is strongly supported by Creer's data³ since the major mass extinctions in the late Ordovician, middle Upper Devonian, end Permian and end Cretaceous coincide with turning points (Fig. 1). Considerable doubt²⁵⁻²⁷ has been cast on whether a magnetic reversal alone would cause a sufficient increase in radiation fluxes but it has recently been suggested that if a solar flare occurs during a reversal it might cause the ozone layer, which shields the Earth's surface from ultraviolet radiation, to become depleted²⁸. Alternative theories²² have suggested a direct relationship between the magnetic field and the well-being of organisms or an indirect relationship through climatic effects.

Correlations between climatic changes and changes in the intensity of the geomagnetic field have been demonstrated and possible linking mechanisms have been discussed^{29,30}. As previously noted there is a correlation between geomagnetic field intensity and rotation rate¹⁷ and Lambeck and Cazenave⁶⁷ have detected a correlation between climate and rotation rate. They have however calculated that the magnitude of the rotational changes is not sufficient to fully explain the associated climatic effects. It is suggested here that magnetic fluctuations cause climatic effects partly as a direct effect and partly through the associated changes in spin velocity, and that the magnetic instabilities at turning points cause climatic instabilities.

The general pattern of mass extinctions is for the communities of stable environments with their great diversity of specialised member species to be most severely affected³¹. Thus offshore benthic communities were more affected than onshore benthic communities^{32,33} and that most complex of all marine associations, the reef community, underwent at each mass extinction a dramatic collapse from which it often took millions of years to recover^{34,35}. The climatic instabilities at turning points were thus probably only triggers, which by causing a few extinctions and/or a reduction in primary production, created a house of cards effect leading to the collapse of ecosystems³¹.

Neither the mid-Carboniferous nor the end Jurassic events were associated with abnormally high extinction rates but Late Pleistocene and Recent extinctions, though dominantly affecting large (that is, specialised) terrestrial vertebrates, are well documented^{36,37}. While man must in all probability take a large share of the blame for these extinctions it may well be that he is only exacerbating an unstable situation.

Climatic history

Apart from the possible climatic instability at turning points the periods of acceleration seem to be characterised by climatic amelioration while periods of deceleration are characterised by climatic deterioration. Thus spin minima were preceded by ice ages with marked seasonal and latitudinal climatic contrast (Fig. 1) while spin maxima were preceded by climatic optima with an equable warm climate. Isotopic evidence^{39,40} (Fig. 1) shows clearly the temperature decline that has taken place, especially at high

latitudes, during the Tertiary. Mesozoic isotope data^{41,42} are less well controlled but indicate warm temperatures even at high latitudes and, though there were fluctuations a trend for temperature increase up to the Late Cretaceous optimum about 80 Myr ago. The Permo-Carboniferous and the Late Ordovician Ice Ages are well documented⁴³ and a recent synthesis³³ has suggested that there was a reduction in climatic gradients during the Silurian and Devonian. The Cretaceous optimum gave rise to the acme of reef development by scleractinian corals and the early Upper Devonian optimum was similarly the acme of the tabulate coral and stromatoporoid reef assemblage³⁴. The scleractinia as a group have declined during the Tertiary⁴⁴ and the poor representation of reef corals in the Carboniferous and Permian may also be a climatic effect. As in the Permian, bryozoans and sponges are an important element of Ordovician carbonate build-up³⁴ formed during a phase of climatic deterioration. A pre-mass extinction diversification has been detected in the Cretaceous and in the Devonian but not in the Permian⁴⁴ which may be a consequence of its different climatic regime.

It is interesting to note that Upper Palaeozoic ice sheets seem to have developed before the mid-Carboniferous aborted event⁴⁵⁻⁴⁷ and this may explain the long time span of the Permo-Carboniferous Ice Age.

Climate and eustasy

Though a change in the length of the day would, for instance, cause a small change in the diurnal temperature inequality⁴⁸, the overall trend evident in the rotational data suggests that the link between climate and spin velocity is not a direct one. Rotational changes may have been accompanied by other changes in the Earth's orbit, which could also have caused slight climatic effects, but the most important control on climate would seem to be sea level⁴⁹. Hays and Pitman postulated⁴⁹ that increased climatic contrast will result from eustatic lowering of sea level and that eustatic raising of sea level will cause 'moderation and stabilisation of climate'. The global Mesozoic transgression and the subsequent Cainozoic regression are well documented⁴⁹⁻⁵³ (Fig. 1). Sea level changes in the Palaeozoic are less clear and estimates based on N. American data⁵³ are clearly affected by tectonic events in the Appalachians. Cruder curves⁵³ (Fig. 1) suggest that there may have been overall transgression from the Late Ordovician to the Late Devonian followed by a regression into the end of the Permian. The Ashgill regression⁵⁴ and the widespread Ordovician-Silurian unconformity⁵⁵ represent the effects of the Late Ordovician eustatic minimum and in the Devonian a series of increasingly penetrative transgressions, which reach a maximum in the Late Devonian, have been recognised⁵⁶. Ice ages are thus a consequence of the increased climatic contrast resulting from regression but the exact timing of the onset of glaciation and the subsequent glacial history will be affected by other factors such as distribution of land masses, atmospheric turbidity and orbital motions. The association of glaciation with aridity⁵⁷ fits well with this theory and the increasing severity of Pleistocene glacial periods³⁷ can be linked to the continued decrease in sea level.

Though orogenic events can cause eustatic changes in sea level⁵² the dominant eustatic control seems to be the volume of oceanic ridges which is itself controlled by plate spreading rates (refs 49, 50, 58 and 59). Thus increased plate generation not only leads to an increasing volume of ocean ridges, a raising of sea level and an amelioration of climate but also correlates with an acceleration of mantle spin velocity and a predominance of normal polarity. Fast Cretaceous and slow Cenozoic spreading rates have been deduced for a number of ridge systems^{49,58}. More detailed work, despite uncertainties due to imprecision of the polarity time scale, suggests that Tertiary spreading rates

decreased gradually up to about 10 to 15 Myr ago, since when there has been a slight increase in spreading rate^{60,61}. This may again suggest an impending turning point. The possible role of tidal and rotational forces in the plate tectonic mechanism has been discussed by several workers⁶²⁻⁶⁶, and, whether the relationships are causal or not, clearly ridge activity and changes in mantle spin velocity and predominant polarity are all related to some unifying process.

A coherent picture—but gaps still remain

Though much work needs to be done to improve our knowledge of the factors considered here, the data present a coherent picture. The Cenozoic, the Late Devonian to Permian and the Ordovician were times of deceleration of mantle spin velocity, of predominantly reversed polarity, of decreasing oceanic ridge activity, of global regression and of climatic deterioration leading to ice ages. By contrast in the Silurian to early Upper Devonian and in the Mesozoic there was acceleration of mantle spin velocity, predominance of normal polarity, increasing ocean ridge activity, global transgression and climatic amelioration. Turning points between phases of different type are marked by magnetic instability, possible climatic instability and by mass extinctions. Aborted turning points occurred in the middle Carboniferous and late Jurassic. Despite the many imperfections and biased nature of the geological record a subtle but distinctive background pattern can thus be detected in it. Whatever the cause may be this pattern is not only a record of surface processes but also of deep seated events some of which may even have taken place within the Earth's core.

This synthesis has a number of important implications. First, it places important new constraints on Earth models. These must be considered especially in discussions of the origin of the geomagnetic field, the mechanisms affecting the Earth's rotation and the driving force for plate tectonics. Second, the pattern can be applied to the Precambrian as an aid to understanding events and processes in the Proterozoic (Whyte, in preparation). Finally it allows us to make some simple predictions about the future geological history of the Earth. Thus not only do we seem to be at present at or close to a turning point (spin minimum) but we can predict that it will be followed by a period lasting about 60 to 90 Myr in which there will be rotational acceleration, a dominantly normal magnetic field, increasing ridge activity, global transgression and climatic amelioration.

Received 4 February; accepted 22 April 1976.

¹ Wells, J. *Nature* 197, 948–50 (1963).

- ² Wells, J. in *Palaeogeophysics* (ed. Runcorn, S. K.) 3–9 (Academic, London, 1970).
- ³ Scrutton, C. T. *Palaeontology* 7, 552–558 (1965).
- ⁴ Runcorn, S. K. *Nature* 204, 823–5 (1964).
- ⁵ Creer, K. M. in *Growth Rhythms and the History of the Earth's Rotation* (eds Rosenberg, G. D. & Runcorn, S. K.) 293–318 (J. Wiley, London, 1975).
- ⁶ Scrutton, C. T. & Hipkin, R. G. *Earth-Sci. Rev.* 9, 259–274 (1973).
- ⁷ Pannella, G., MacClintock, C. & Thompson, M. N. *Science* 162, 792–796 (1968).
- ⁸ Pannella, G. *Astrophys. Space Sci.* 16, 212–237 (1972).
- ⁹ Pannella, G. & MacClintock, C. *Palaeontol. Soc. Mem.* 2, 64–80 (1968).
- ¹⁰ Scrutton, C. T. in *Palaeogeophysics* (ed. Runcorn, S. K.) 11–16 (Academic, London, 1970).
- ¹¹ Berry, W. B. N. & Barker, R. M. *Nature* 217, 938–939 (1968).
- ¹² Berry, W. B. N. & Barker, R. M. in *Growth Rhythms and the History of the Earth's Rotation* (eds Rosenberg, G. D. & Runcorn, S. K.) 9–25 (J. Wiley, London, 1975).
- ¹³ Johnson, G. A. L. & Nudds, J. R. in *Growth Rhythms and the History of the Earth's Rotation* (eds Rosenberg, G. D. & Runcorn, S. K.) 27–42 (J. Wiley, London, 1975).
- ¹⁴ McGugan, A. *Abstr. Ann. Meeting geol. Soc. Am.* 145 (1967).
- ¹⁵ Muller, P. M. & Stephenson, F. R. in *Growth Rhythms and the History of the Earth's Rotation* (eds Rosenberg, G. D. & Runcorn, S. K.) 459–534 (J. Wiley, London, 1975).
- ¹⁶ Strangway, D. W. *History of the Earth's Magnetic Field* (McGraw-Hill, New York, 1970).
- ¹⁷ Yukutake, T. J. *Geomag. Geoelec.* 25, 195–212 (1973).
- ¹⁸ Rochester, M. G. *Trans. Am. geophys. Union* 54, 769–781 (1973).
- ¹⁹ Tarling, D. H. in *Growth Rhythms and the History of the Earth's Rotation* (eds Rosenberg, G. D. & Runcorn, S. K.) 397–412 (J. Wiley, London, 1975).
- ²⁰ Irving, E. & Pullaiah, G. *Earth-Sci. Rev.* 12, 35–64 (1976).
- ²¹ Cox, A. *Science* 163, 237–245 (1969).
- ²² Hays, J. D. *Bull. geol. Soc. Am.* 83, 2433–47 (1971).
- ²³ Uffen, R. J. *Nature* 198, 143 (1963).
- ²⁴ Simpson, J. F. *Bull. geol. Soc. Am.* 77, 197–204 (1969).
- ²⁵ Black, D. I. *Earth planet. Sci. Lett.* 3, 225–236 (1967).
- ²⁶ Waddington, C. J. *Science* 158, 912–5 (1967).
- ²⁷ Harrison, C. G. A. *Nature* 217, 46–47 (1968).
- ²⁸ Reid, G. C., Isaksen, I. S. A., Holzer, T. E. & Crutzen, P. J. *Nature* 259, 177–179 (1976).
- ²⁹ Wollin, G. et al. *Nature* 242, 34–36 (1973).
- ³⁰ Harrison, C. G. A. & Prospero, J. M. *Nature* 250 563–6 (1974).
- ³¹ Bretsky, P. W. & Lorenz, D. M. *Bull. geol. Soc. Am.* 81, 2449–56 (1970).
- ³² Bretsky, P. W. *Palaeogeog. Palaeoclim. Palaeoecol.* 6, 45–59 (1969).
- ³³ Walker, K. R. J. *Paleontol.* 46, 82–93 (1972).
- ³⁴ Newell, N. D. *Sci. Am.* 226, 54–56 (1972).
- ³⁵ Boucot, A. J. *Developments Palaeontol. Stratigr.* 1, 427 (1975).
- ³⁶ Martin, P. S. & Wright, H. E. *Pleistocene Extinctions: the Search for a Cause* (Yale Univ. Press, Yale, 1968).
- ³⁷ Van Valen, L. *Proc. N. Am. Palaeontol. Convention* 469–485 (1969).
- ³⁸ Cutbill, J. L. & Funnell, B. M. in *The Fossil Record* (eds Harland, W. B. et al.) 791–820 (Geol. Soc. Lond., London, 1967).
- ³⁹ Savin, S. M., Douglas, R. G. & Stehli, F. G. *Bull. geol. Soc. Am.* 86, 1499–1510 (1975).
- ⁴⁰ Devereux, I. *New Zealand J. Sci.* 10, 988–1011 (1967).
- ⁴¹ Bowen, R. *Methods Geochem. Geophys.* 2, 265 (1966).
- ⁴² Clayton, R. N. & Stevens, G. R. *Tuataria* 16, 3–7 (1968).
- ⁴³ Wright, A. E. & Moseley, F. *Geol. J. Spec. Issue* 6, 320 (1975).
- ⁴⁴ Thompson, K. S. *Nature* 261, 578–580 (1976).
- ⁴⁵ Harrington, H. J. *Am. Assoc. Petrol. Geol. Bull.* 46, 1773–1814 (1962).
- ⁴⁶ Frakes, L. A. & Crowell, J. C. *Bull. geol. Soc. Am.* 80, 1007–42 (1969).
- ⁴⁷ Frakes, L. A. & Crowell, J. C. *Bull. geol. Soc. Am.* 81, 2261–86 (1976).
- ⁴⁸ Lovenburg, M. F., Dell, C. I. & Johnson, M. J. S. *Bull. geol. Soc. Am.* 83, 3529 (1972).
- ⁴⁹ Hays, J. D. & Pitman, W. C. III *Nature* 246, 18–22 (1973).
- ⁵⁰ Hallam, A. *Am. J. Sci.* 261, 397–423 (1963).
- ⁵¹ Hallam, A. *Earth-Sci. Rev.* 5, 45–68 (1969).
- ⁵² Grasty, R. L. *Nature* 216, 779–80 (1967).
- ⁵³ Wise, D. U. *Mem. geol. Soc. Am.* 132, 87–100 (1972).
- ⁵⁴ Berry, W. B. N. & Boucot, A. T. *Bull. geol. Soc. Am.* 841, 275–284 (1973).
- ⁵⁵ Dennison, J. M. in *The Ordovician System* (ed. Bassett, M. G.) 107–120 (University of Wales Press and Na. Mus. of Wales, Cardiff, 1976).
- ⁵⁶ House, M. R. *Proc. Yorks. geol. Soc.* 40, 233–287 (1975).
- ⁵⁷ Fairbridge, R. W. in *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 503–515 (Academic, London, 1973).
- ⁵⁸ Armstrong, R. L. *Nature* 221, 1042–43 (1969).
- ⁵⁹ Vine, F. J. in *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 831–839 (Academic Press, London, 1973).
- ⁶⁰ Vogt, P. R. & Avery, O. E. J. *geophys. Res.* 79, 363–389 (1974).
- ⁶¹ Fleming, N. C. & Roberts, D. G. *Nature* 243, 19–22 (1973).
- ⁶² Howell, B. F. J. *geophys. Res.* 75, 2769–72 (1970).
- ⁶³ Hughes, T. *Tectonophysics* 18, 215–30 (1973).
- ⁶⁴ Moore, G. W. *Am. Assoc. Petrol. Geol. Bull.* 59, 1020–1 (1975).
- ⁶⁵ Torbett, M. V. *Tectonophysics* 30, T17 (1976).
- ⁶⁶ Bostrom, R. C. *The Moon* 15, 109–119 (1976).
- ⁶⁷ Lambeck, K. & Cazenave, A. *Geophys. J. R. astr. Soc.* 46, 555–73. (1976).

Biogenic lipids in particulates from the lower atmosphere over the eastern Atlantic

Bernd R. T. Simoneit

Institute of Geophysics and Planetary Physics, University of California, Los Angeles, California 90024

R. Chester

Department of Oceanography, University of Liverpool, Liverpool, UK

G. Eglinton

School of Chemistry, University of Bristol, Bristol, UK

Higher plant wax is demonstrated in aeolian dusts, showing the potential of aeolian transport in contributing significant amounts of terrestrial organic lipids to the marine environment over geological time periods.

THE importance of aeolian particulate transport from the continents to the marine environment has been examined mainly through investigations of mineral and trace element contents of sediments and particulate matter in the water column^{1–4}. This transport mechanism, however, has been

Table 1 General aeolian dust collection data and sample descriptions

Sample*	Date collected	Ship's tracks†	Dust colour	Dust loading ($\mu\text{g m}^{-3}$)	Wind system	Potential dust source
1	Apr. 72	34°44'N, 13°38'W 28°30'N, 15°54'W	Brown	0.18	Westerlies/ N.E. Trade Boundary	N.W. Europe N. Africa
2	Mar. 71	21°41'N, 17°41'W 14°04'N, 18°07'W	Beige	36	N.E. Trades	Sahara
3	July 72	17°47'N, 17°42'W 19°24'N, 17°40'W	Beige	~20	N.E. Trades	Sahara
4	Dec. 74	03°44'N, 12°00'W 08°50'N, 15°44'W	Grey-brown	9.6	N.E. Trades	Sahara
5	Dec. 74	01°14'S, 08°01'W 03°44'N, 12°00'W	Dark grey	0.4	N.E. Trades	Sahara
6	July 72	07°12'S, 04°02'W 02°37'S, 07°42'W	Brown-black	0.18	S.E. Trades	Southern Africa
7	July 72	11°52'S, 00°29'W 07°12'S, 04°02'W	Dark brown	0.08	S.E. Trades	Southern Africa
8	Sept. 74	09°40'S, 01°31'W 14°42'S, 02°06'E	Dark brown	1.4	S.E. Trades	Southern Africa
9	July 72	22°26'S, 07°58'E 17°01'S, 03°30'E	Brown	0.29	S.E. Trades	Southern Africa
10	Sept. 74	14°42'S, 02°06'E 19°26'S, 05°47'E	Beige-brown	2.0	S.E. Trades	Southern Africa
11	Nov. 71	29°03'S, 13°22'E 25°06'S, 09°59'E	Black	5.6	S.E. Trades	Ships
12	July 72	30°03'S, 31°03'E 24°24'S, 24°07'E	Grey-brown	4.0	Variables	Southern Africa

*Samples 1 (TAF 5), 3 (TAF 71), 6 (TAF 65), 7 (TAF 64), 9 (TAF 62) and 12 (TAF 57) see reference 10; 2 (E2) see ref. 11; 4 (DC 2), 5 (DC 1), 8 (DC 4) and 10 (DC 5) Cross, unpublished results; and 11 (AH 3) see ref. 12.

†Indicates the position of the ship at the start and finish of each sampling run.

known since Darwin's time⁵. Biological material transported from the continents has been identified as discrete particles such as freshwater organisms, fungal hyphae, spores, plant fragments and insects^{2,4,6,7}. Terrigenous lipids such as the prevalent vascular plant waxes have been described as components of some aeolian dusts. Here we discuss the occurrence, isolation and characterisation of terrigenous lipids in aeolian dusts from the eastern Atlantic. Such lipids have also been found in aeolian dust from other oceanic areas⁸ and could be correlated with sedimentary lipids from deep-sea areas which have a large input of aeolian mineral components⁹. Lipids derived from terrigenous (higher plant) sources are primarily characterised by the distributions of the *n*-alkanes from *n*-C₂₃ to *n*-C₃₅ (odd carbon number predominance), the *n*-fatty acids from *n*-C₂₂ to *n*-C₃₂ (even carbon number predominance) and *n*-fatty alcohols from *n*-C₂₂ to *n*-C₃₂ (even carbon number predominance)⁹. Lipids derived from microbiota of either marine or lacustrine environments are characterised by the absence of these higher molecular weight homologues (> C₂₅) and the presence of *n*-alkanes ranging from *n*-C₁₃ to *n*-C₂₃ with a major maximum at C₁₇ and no significant carbon number predominance, and *n*-fatty acids ranging from *n*-C₁₂ to *n*-C₂₀ with a major maximum at C₁₆ and an even predominance⁹.

Collection and extraction of samples

Small dust samples were obtained from the research collection of the Department of Oceanography of the University of Liverpool. The samples had been stored in glass vials since completion of the inorganic analyses^{10,11}. The net-sampling procedure used here has been applied extensively for inorganic analyses of airborne particulates^{4,11,12}. The collection efficiency of the nets has been investigated in detail by various authors^{4,6,13} and it was found that such nets collect only about 50% of the < 2- μm size fraction and about 70% of the < 4- μm size fraction. The dust particles adhere to the net by concurrent wetting with sea spray and are washed salt-free afterwards⁹⁻¹².

The dust samples were extracted with two aliquots of toluene and methanol (3:1) for lipid analysis, and the extracts were concentrated on a rotary evaporator. After capillary gas chromatographic (GC) and gas chromatography-mass spectrometric (GC/MS) analyses all total extracts were treated with

diazomethane in ether and re-analysed by GC and GC/MS. Some extracts were further treated with *N,O*-bis-trimethylsilylacetamide (BSA) reagent to derivatise alcohols to trimethylsilyl (TMS) ethers and then subjected to GC and GC/MS analysis. The large sample extracts were separated by thin-layer chromatography (TLC) on silica gel with hexane-ether (9:1) as eluant into fractions corresponding to the retention times of hydrocarbons, esters and alcohols. These fractions were also subjected to instrumental analysis. The approximate yields of total lipids were estimated by weighing a 1-to-5- μl aliquot of total extract, after the solvent was allowed to evaporate, on a Cahn microbalance. These values were usually somewhat higher than those obtained by the sums of the integrations of the GC results from the hydrocarbon, acid ester and alcohol fractions. The instrumental analyses (GC and GC/MS) and the resultant detailed data have been described elsewhere^{8,9,14}.

The general collection data and descriptions for all samples are given in Table 1, and the sampling sites are shown on the map in Fig. 1. This sample suite represents a transect down the Atlantic Ocean from Spain past the tip (Cape of Good Hope) of South Africa (see Fig. 1). The beige Saharan dusts (samples 1 to 3) yielded relatively low amounts of solvent-soluble lipids. The dusts from the more tropical and the more vegetated environments (for example, samples 4 to 8) were grey to grey-brown and yielded larger amounts of solvent-soluble lipids.

The yields and analytical results for the *n*-alkanes and *n*-carboxylic acids are given in Table 2, together with some representative organic carbon values. The *n*-alkanes exhibited maxima at mainly *n*-C₂₉, *n*-C₃₁, and *n*-C₂₇, with strong odd-to-even carbon number predominances (see carbon preference index (CPI) in Table 2). The *n*-fatty acids maximised at *n*-C₁₈ and either *n*-C₂₄ or *n*-C₂₆, with strong even-to-odd carbon number predominances (see CPI in Table 2). The *n*-alkanes and *n*-fatty acids of all samples were distinguished from *iso*-, *anteiso*- and isoprenoidal compounds by coinjection of suitable standards on capillary GC, mass fragmentography and interpretation of individual low resolution mass spectra from GC/MS analyses. The *n*-alcohols exhibited a maximum at *n*-C₂₈, with a strong even-to-odd carbon number predominance (see CPI in Table 2). The distribution diagrams for all these lipid fractions can be found elsewhere¹⁴.

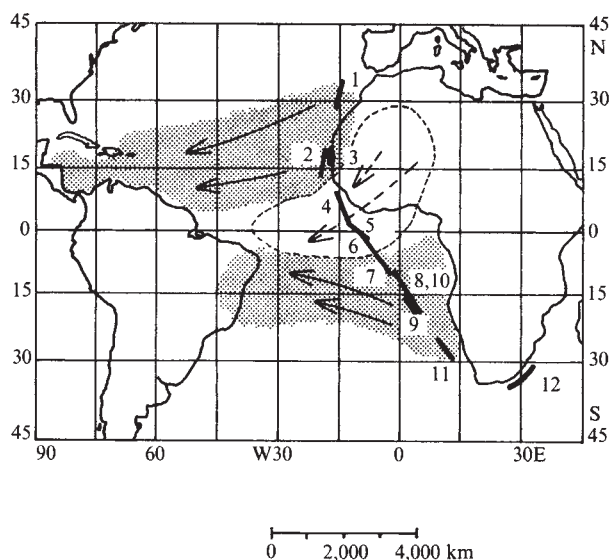


Fig. 1 Map showing the aeolian dust sampling locations and prevalent wind patterns (thick line, ship's track during aeolian dust collection; solid arrows, approximate extent of trade winds; dashed arrows, Harmattan wind).

Origin of samples

Sample 1 was taken off the coast of Morocco in the westerlies/north-east trades boundary zone and probably contains material derived from a variety of sources, including western Europe (for example, Spain) and the North African deserts. The extracted mixture consisted mainly of free fatty acids ranging from $n\text{-C}_{14}$ to $n\text{-C}_{20}$, with a maximum at $n\text{-C}_{16}$ and predominantly even homologues, and a minor amount of $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes. This acid distribution is a typical lacustrine and/or marine pattern⁹. Samples 2 and 3 consist mainly of the trade wind flux of Saharan material. The lipid yields were different for the two samples, but the distributions of homologues were essentially identical.

Samples 4 and 5 were collected off West Africa and probably represent mainly Saharan and West African material carried by the Harmattan winds (see Fig. 1)¹¹. Both lipid extracts exhibited distributions of homologues of predominantly $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes and $n\text{-C}_{22}$ to $n\text{-C}_{30}$ fatty acids, which are typical of higher plant wax¹⁵ and minor amounts of $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids attributable to either a lacustrine or marine origin^{9,16}.

The remaining samples (6 to 10 and 12) represent fallout from the south-east trades (see Fig. 1), deriving material

mainly from the soils of southern Africa, the Namib and Kalahari deserts, and probably from the Lower Guinea area of Africa. Samples 9 and 10 were taken in the same area but at different seasonal times. The distributions of lipid homologues for these two samples were similar and the distributions of homologues for all the samples consisted of predominantly $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes and $n\text{-C}_{22}$ to $n\text{-C}_{30}$ fatty acids, and lesser amounts of $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids.

The extracts of several samples (1, 6, 8 and 9) contained mono-unsaturated fatty acids (mainly $\text{C}_{18:1}$ and some $\text{C}_{16:1}$) in significant amounts. Trace amounts of triterpenoidal compounds were detected in the samples with a high plant wax component (such as 4, 5, 6, and 8) but not in the samples derived from more arid terrain (such as 1 to 3). Diterpenoidal compounds which have been proposed as terrigenous markers¹⁷ were not detected in any aeolian dust extracts examined thus far. Polynuclear aromatic hydrocarbons have also not been detected as any significant components (GC/MS detection limit approximately 0.1 p.p.m. per component). The problems of plasticiser and shipboard contamination were not serious and have been described in detail^{8,14,18}. Sample 11 represents an example of shipboard contamination where the lipids exhibited a general 'hump' distribution, typical of petroliferous material.

The n -alkanes, n -alkanoic acids and n -alcohols in the lipids of these aeolian dusts correlate well with terrigenous sources.

The n -alkanes observed in these dusts have essentially no homologues lower than about C_{22} and their distribution patterns corroborate a terrigenous origin from higher plant waxes^{15,19-21}. The extreme fragility of the plant wax protrusions has been described (ref. 22 and references cited therein). Wax particles are easily sloughed off from plant surfaces and can become airborne. There are not enough data points at this time to ascribe a significance or specific plant sources to the shifts of the maxima in the n -alkane distributions from $n\text{-C}_{27}$ in the North Atlantic (1), to $n\text{-C}_{27} \cong n\text{-C}_{29}$ (2, 3), to $n\text{-C}_{29}$ in the Inter-Tropical Convergence Zone (4 to 6), to $n\text{-C}_{29} \cong n\text{-C}_{31}$ (8), and to $n\text{-C}_{31}$ in the South Atlantic (9, 10) (see Table 2).

The n -alkanoic acid distributions of all the dusts exhibit a typically lacustrine and/or marine pattern, with maxima at C_{16} and superimposed on that a higher plant distribution pattern. Such fatty acid distributions have been described by many authors for sediments (see for example, ref. 23). This observation fits in part with Kolbe's proposal¹⁶ that aeolian dusts have a component derived from dessicated lacustrine muds. However, since the sampling nets were wet with sea-spray, some of the $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids may also have a marine origin. Normal carboxylic acids with maxima at C_{24} or C_{26} and a strong even-to-odd predominance have been used as terrigenous

Table 2 Analytical results for aeolian dusts

Sample	Organic carbon (%) [*]	Total lipids		n -Alkanes			n -Fatty acids			n -Alcohols		
		(~ μg per g of dust)	(ng per m^3 of air)	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no. [‡]	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no. [‡]	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no.
1	12	750	0.13	10	10.2	27	740	n.d.	16	n.d.	—	—
2	2	130	4.7	40	1.4	25, 29	20	6.1	16, 24	70	4.6	28
3	2	460	10.0	400	7.5	27 $\approx$ 29	35	22	16, 24	n.d.	—	—
4	7	850	8.0	340	5.0	29	160	11.6	16, 20	240	2.5	28
5	12	2,400	1.0	860	3.2	29	900	7.5	16, 24	n.d.	—	—
6	10	9,800	1.8	2,200	5.6	29	3,400	14	16, 24	2,600	4.2	28
7	8	370	0.03	45	6.0	29 $\approx$	n.d.	—	—	n.d.	—	—
8	8	2,700	3.8	1,150	5.2	29 $\approx$ 31	400	4.1	26	650	3.3	28
9	8	270	0.08	120	6.5	29 $\approx$ 31	40	26	16, 26	n.d.	—	—
10	8	100	0.2	40	5.2	31	12	7.5	16, 26	n.d.	—	—
11	80	n.d.	n.d.	n.d.	—	hump	n.d.	—	—	n.d.	—	—
12	9	3,050	12.0	1,050	7.5	27	260	8.4	16, 26	n.d.	—	—

^{*}Ref. 12 (values for samples taken in the same area).

[†]Carbon preference index (CPI)^{23,24} was calculated for all homologues from C_{10} to C_{35} .

[‡]The major distribution maximum is underscored.

n.d., Not determined.

markers²⁴⁻²⁶ and these homologues from the dusts thus probably also represent a higher plant wax component. A shift of the maxima in the *n*-fatty acid distributions, analogous to the *n*-alkanes, was observed from *n*-C₂₄ in the North Atlantic samples to *n*-C₂₆ in the Mid- to South Atlantic samples. Again, no specific plant sources can be assigned to these limited data.

The higher molecular weight *n*-alcohol distributions observed for the dust samples (see Table 2) closely resemble the distributions of alcohols from plant wax. These consist of predominantly even-carbon-numbered homologues from *n*-C₁₆ to *n*-C₃₆, usually with a maximum at *n*-C₂₈^{15,21}. The presence of traces of triterpenoids in these dusts indicates that these compounds can be derived from a terrigenous source. The amounts of mono-unsaturated fatty acids (*n*-C_{16:1} and *n*-C_{18:1}) when considered with the distribution patterns of the saturated *n*-fatty acids < C₂₀, indicate a lacustrine and/or marine origin for these components. The presence of these labile compounds in the dusts also indicates that the destruction (photo-oxidation) of organic matter by ultraviolet radiation during transit is limited. The variations in the homologue distributions of the dust lipids from the relatively close geographical areas (for example, 9 and 10, 2 and 3, 5 and 6) may probably be accounted for by seasonal variations of plant source material and wind pattern change.

Anthropogenic components were found in dusts derived from urban and industrialised areas⁸. The hydrocarbons of aeolian dusts collected near urban centres have quite a different compound distribution, attributable mainly to petroleum product spillage and combustion (see for example refs 27, 28). The *n*-alkanes of urban air particulates are present as minor components as part of a general 'hump' typical of petroleum and they exhibit no carbon number predominance. On the other hand, the *n*-alkanes of the oceanic dusts are present as the major components and exhibit a very strong odd-to-even carbon number predominance. The sampling procedure using nets (meshes), however, does not collect aerosol particles, which can be major components of urban atmospheres (ref. 29 for example). Some oceanic dusts collected in proximities of urbanised areas (for example, inland sea off Japan⁸) also exhibit a 'hump' in the hydrocarbon fraction.

The sedimentation rates in the North Atlantic Ocean for the aeolian components off West Africa have been estimated to be about 0.6 mm per 1,000 yr (ref. 4). This figure is in good agreement with those reported by Goldberg and Griffin³⁰ for the equatorial regions of the Atlantic. Observed sedimentation rates adjacent to the Canary and Cape Verde Islands, based on ¹⁴C dates, range from 2-3 cm per 1,000 yr (ref. 31). A more recent input estimate of organic matter from dust to the ocean has been proposed³². It was found that during North African dust storms, extremely large amounts (> 10,000 µg m⁻³ at the coast) of dust are transported out to sea along about a 100 km stretch of coastline, thus also injecting a large organic component (about 20,000 tonnes of organic carbon per storm). Thus aeolian influx to the marine environment occurs in random major events and continuously in lower amounts.

The bulk of the lipid matter of aeolian dusts represents higher plant wax¹⁵ and fatty acids from lacustrine microbiota^{4,16} or possibly from sea-spray. It should be noted that the lipid yields for these samples are low when compared to the organic carbon contents of other samples from similar areas (see Table 2). No total carbon values could be determined for this sample suite. This difference may be partially due to a particulate carbon component from ships and a loss of volatile lipids during the analysis. Higher lipid yields against organic carbon were obtained for samples collected at the coastline of West Africa³². Work is proceeding to understand the budget of the influx of aeolian organic carbon compounds to the oceanic environment. It is, however, apparent that the input of aeolian material to certain sedimentary areas can be significant over geological time periods.

We thank Dr S. R. Aston and Mr D. Cross for providing some of the dust samples and Dr R. Philp for use of GC/MS facilities. Financial support in part from the National Aeronautics and Space Administration and the National Oceanographic and Atmospheric Administration is gratefully acknowledged.

Received 14 February; accepted 29 April 1977.

- 1 Parkin, D. W., Delany, A. C. & Delany, Audrey C. *Geochim. Cosmochim. Acta* **31**, 1311-1320 (1967).
- 2 Chester, R. & Elderfield, H. *New Scientist* **47**, 432-434 (1970).
- 3 Chester, R. in *Nobel Symposium 20: The Changing Chemistry of the Oceans* (eds Dyrssen, D. & Jagner, D.) 291-305 (Wiley, New York, 1972).
- 4 Delany, A. C., Delany, A. C., Parkin, D. W., Griffin, J. J., Goldberg, E. D. & Reimann, B. E. F. *Geochim. Cosmochim. Acta* **31**, 885-909 (1967).
- 5 Darwin, C. *Q. Jl Geol. Soc. Lond.* **2**, 26-30 (1846).
- 6 Goldberg, E. D. *Comments in Geophys. Earth Sci.* **1**, 117-132 (1971).
- 7 Rex, R. W. & Goldberg, E. D. in *The Sea*, **1** (ed. Hill, M. N.), 295-304 (Interscience, New York, 1962).
- 8 Simoneit, B. R. T. & Eglinton, G. in *Adv. Org. Geochem. 1975* (eds Gómez Angulo, J. A. & Campos, R.) (in the press).
- 9 Simoneit, B. R. T. thesis, Univ. Bristol (1975).
- 10 Chester, R. & Stoner, J. H. *Marine Chem.* **2**, 157-188 (1974).
- 11 Chester, R., Elderfield, H., Griffin, L. R. & Padgham, R. C. *Marine Geol.* **13**, 91-105 (1972).
- 12 Aston, S. R., Chester, R., Johnson, L. R. & Padgham, R. C. *Marine Geol.* **14**, 15-28 (1973).
- 13 Parkin, D. W., Phillips, D. R., Sullivan, R. A. L. & Johnson, L. R. *Q. Jl R. Met. Soc.* **98**, 798-808 (1972).
- 14 Simoneit, B. R. T. *Symposium on Concepts in Marine Organic Chemistry, Edinburgh, Sept. 1976, Marine Chem.* (in the press).
- 15 Eglinton, G. & Hamilton, R. J. in *Chemical Plant Taxonomy* (ed. Swain, T.), 187-217 (Academic, New York, 1963).
- 16 Kolbe, R. W. *Science* **126**, 1053-1056 (1957).
- 17 Simoneit, B. R. T., *Geochim. Cosmochim. Acta* **41**, 463-476 (1977).
- 18 Moyers, J. L., Duce, R. A. & Hoffman, G. L. *Atmos. Environ.* **6**, 551-556 (1972).
- 19 Douglas, A. G. & Eglinton, G. in *Comparative Phytochemistry* (ed. Swain, T.), 57-77 (Academic, New York, 1966).
- 20 Caldicott, A. B. & Eglinton, G. in *Phytochemistry, III* (ed. Miller, L. P.), 162-194 (Van Nostrand-Reinhold, New York, 1973).
- 21 Kolattukudy, P. E. & Walton, T. J. in *Progress in the Chemistry of Fats and Lipids* (ed. Holman, R. T.), Vol. 13, Part 3, 121-175 (Pergamon, 1972).
- 22 Holloway, P. J. in *Ecology of Leaf Surface Micro-organisms* (eds Preece, T. F. & Dickenson, C. H.), 49 (Academic, 1971).
- 23 Eglinton, G., Maxwell, J. R. & Philp, R. P. in *Adv. Org. Geochem. 1973* (eds Tissot, B. & Biennier, F.), 941-961 (Editions Technip., Paris, 1974).
- 24 Hitchcock, C. & Nichols, B. W. *Plant Lipid Biochemistry* (Academic, London, 1971).
- 25 Kvenvolden, K. A. J. *Am. Oil Chem. Soc.* **44**, 628-636 (1967).
- 26 Markley, K. S. *Fatty Acids*, 2nd ed., 5 vols. (Interscience, New York, 1968).
- 27 Cukor, P., Ciccio, L. L., Lanning, E. W. & Rubino, R. L. *Environ. Sci. Technol.* **6**, 633-637 (1972).
- 28 Hauser, T. R. & Pattison, J. N. *Environ. Sci. Technol.* **6**, 549-555 (1972).
- 29 Lee, R. E., Jr. *Science* **178**, 567-575 (1972).
- 30 Goldberg, E. D. & Griffin, J. J. *geophys. Res.* **69**, 4293-4302 (1964).
- 31 Rothe, P. *Mar. Geol.* **14**, 191-206 (1973).
- 32 Lepple, F. K. & Brine, C. J. *geophys. Res.* **81**, 1141-1147 (1976).
- 33 Cooper, J. E. & Bray, E. E. *Geochim. Cosmochim. Acta* **27**, 1113-1127 (1963).
- 34 Kvenvolden, K. A. *Nature* **209**, 573-577 (1966).

letters to nature

Upper limits to X-ray emission from nova Vulpeculae 1976

Two recent optical novae have been observed to radiate a substantial flux of medium energy X rays (nova Monocerotis¹, and A1524-61 (ref. 2)), the X-ray luminosity in both cases reached a maximum at the same time as the optical

luminosity and was over 100 times greater. In contrast, an unsuccessful search was made by Hoffman *et al.*³ for X-ray emission from nova Cygni 1975 and the ratio of X-ray to optical luminosity in that case was found to be less than 10⁻⁴. Brecher and Morrison⁴ have suggested that the large differences in this ratio might be related to the rate of mass exchange in the binary system, believing that the X-ray

Table 1 X-ray upper limits

Mid-time (1976)	Orbit number	X-ray flux, L_x ($\text{erg cm}^{-2} \text{s}^{-1}$)
Oct 20.86	11184	$< 5.5 \times 10^{-9}$
20.92	11185	$< 3.7 \times 10^{-9}$
20.99	11186	$< 3.3 \times 10^{-9}$
21.07	11187	$< 3.4 \times 10^{-9}$
21.13	11189	$< 3.5 \times 10^{-9}$
21.20	11190	$< 3.5 \times 10^{-9}$
21.27	11191	$< 3.3 \times 10^{-9}$
21.33	11192	$< 3.2 \times 10^{-9}$
21.39	11193	$< 3.2 \times 10^{-9}$
21.53	11194	$< 3.2 \times 10^{-9}$

emission arises from the shock heating of dense circumstellar material by the nova ejecta. Only systems with a high density in the accretion flow, such as recurrent novae, will therefore generate large X-ray luminosities.

There is little observational evidence to test a relationship between recurrent activity and X-ray emission in optical novae; consequently, a search has been made through Ariel V data for times at which an optical nova was within the 17° field of view of the Rotation Modulation Collimator (Experiment A). Ten orbits of observations in October 1976 coincided with the maximum of nova Vulpeculae which was within the field of view from October 20.83 UT to October 21.53 UT. The optical outburst of this nova was detected by Alcock³ at a visual magnitude of 6.5 on October 21.764 UT. Gorynya⁶ reported a photographic magnitude of 8.8 on 20.696 UT which, together with a pre-outburst measurement by Shao⁷ of $m_{\text{phot}}=18.3$, indicated that the nova was near maximum during the Ariel V observations, and hence a likely source of X-ray emission.

No significant X-ray flux was detected and so an upper limit to the ratio of X-ray to optical luminosity of $\sim 10^{-1}$ can be set from these measurements, suggesting that nova Vulpeculae 1976 may be similar to nova Cygni 1975 in this respect.

Table 1 lists the upper limits to the X-ray flux from nova Vulpeculae for each orbit, together with the mid-time of each data integration. The upper limits are uncertain to within a factor of two due to the non-uniform spectral response of the detectors which are sensitive in the energy range 2.94–7.60 keV.

Table 2 is a compilation of the measurements of X-ray emission from optical novae and contains the ratio of X-ray to optical luminosity and the available evidence for recurrent

Table 2 X-ray emission from optical novae

Name	L_x/L_{opt}	Evidence for previous optical activity
A1524-61	$\sim 1.5 \times 10^2$	None reported, probably too faint for plate searches
nova Monocerotis 1975	$\sim 10^3$	Previous outburst, reported by Eachus ⁸
nova Cygni 1975	$< 10^{-4}$	No search reported
nova Vulpeculae 1976	$< 10^{-1}$	No previous activity back to 1896 in Harvard plate collection

optical activity. A search has been made by L. J. Chaisson (personal communication) in the Harvard Plate Collection for previous outbursts from nova Vulpeculae but none have been detected back to 1896, consequently the small amount of data available at present in Table 2 does not conflict with the hypothesis that X-ray emission accompanies recurrent activity in optical novae. The study of optical novae would clearly benefit from more X-ray observations at the period of maximum light.

I am grateful to Dr L. J. Chaisson for communicating the results of the Harvard plate search and to Dr J. L. Culhane for helpful discussions.

A. M. CRUISE

Department of Physics,
University College London,
Mullard Space Science Laboratory,
Dorking,
Surrey, UK

Received 25 March; accepted 26 April 1977.

- ¹ Elvis, M., Griffiths, R. E., Turner, M. J. K. & Page, C. G. *IAU Circ.* 2814 (1975).
- ² Murdin, P., Griffiths, R. E., Pounds, K. A., Watson, M. G. & Longmore, A. T. *Mon. Not. R. astr. Soc.* 178, 27 (1977).
- ³ Hoffman, J. A., Lewin, W. H. G., Brecher, K., Buff, J., Clark, G. W., Joss, D. C. & Matilsky, T. *Astrophys. J. Lett.* (1976).
- ⁴ Brecher, K. & Morrison, D. *B.A.A.S.* 7, 58 (1975).
- ⁵ Alcock, G. E. D. *IAU Circ.* 2997 (1975).
- ⁶ Gorynya, N. *IAU Circ.* 3008 (1975).
- ⁷ Shao, C. Y. *IAU Circ.* 2998 (1975).
- ⁸ Eachus, L. J. *IAU Circ.* 2823 (1975).

Electron-positron flux at the surface of the pulsar in the Crab

LARGE magnetic fields at the surface of neutron stars favour the formation of a condensed phase of electrons and positrons. It is possible to account for the narrow γ -ray line recently observed in the Crab spectrum from this phase with a flux of electrons and positrons at the surface of the neutron star in the Crab consistent with a recent model.

Leventhal, MacCallum and Watts¹ have, with a balloon-borne γ -ray telescope, recently detected a line feature at 400 keV emanating from the Crab nebula. This energy has the correct magnitude of red shift to be due to two-photon electron-positron (e^-e^+) annihilations at the surface of the pulsar in the Crab. About 10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ are detected, which if they are emitted isotropically imply that the rate of annihilations at the pulsar is at least 10^{41}s^{-1} . If the annihilations take place in the polar gap region alone, and the photons travel along the magnetic field lines, this number is reduced to about 10^{40}s^{-1} . The most surprising feature of Leventhal *et al.* observations is the width of the line which is determined by the resolution of the detector to be less than 3 keV. One expects the spectrum over which the annihilation photons are distributed to be determined by the transverse momentum of the electrons and positrons in the magnetic field. For a field of 10^{12}G , this may be determined to be about 50 keV. Fields less than 10^{12}G are considered unacceptable at the surface of the pulsar in the Crab.

A possible mechanism to have narrow lines from e^-e^+ annihilation is for the annihilation to be stimulated by annihilation photons. This leads to the amplification of photon density near a particular energy. Stimulated annihilation processes require population inversion. In the present context this means that the electron and positron fluids be nearly degenerate—the density be such that the Fermi energy is much higher than the temperature.

The large magnetic field at the surface of neutron stars favours the condensation of electrons and positrons into rod-shaped droplets. The density in the droplets is high enough to obtain degeneracy.

Following Ruderman's² calculations of the properties of matter in superstrong magnetic fields, Chui³ has variationally estimated the binding energy of an electron-hole droplet in large magnetic fields in a semiconductor. One can equally well use this estimate for an e^-e^+ fluid. The variational estimate of the binding energy is

$$E_b = -13.4 (a_0/\rho_0)^{2/3} e^2/a_0 \quad (1)$$

where a_0 is the Bohr radius and ρ_0 is the cyclotron radius. The

radial extent of the electron or positron wave function transverse to the field

$$r \simeq 1.1 a_0 (\rho_0/a_0)^{2/3} \quad (2)$$

the linear density of the drop along the magnetic field

$$l^{-1} \simeq 1.72 a_0^{-1} (a_0/\rho_0)^{2/3} \quad (3)$$

and the Fermi energy of the one-dimensional fluid is

$$E_f = (h^2/2m)l^{-2} \quad (4)$$

For $B = 10^{12}$ G, one gets $\rho_0 \simeq 4.9 \times 10^{-2} a_0$, $r \simeq 0.15 a_0$, $l \simeq 0.075 a_0$, $E_b \simeq -2.75$ keV, and $E_f \simeq 3.9$ keV. Since the temperature at the surface of the neutron star in the Crab is estimated to be less than 0.5 keV, I concluded that if a steady state with the above parameters is attained, the droplet is indeed degenerate.

I shall next consider whether a steady state can be reached. The electrons and positrons arrange themselves in parallel rods among which there will be a weak residual van der Waals attraction which may or may not be sufficient to bind them to each other. If they bind, the rods will be spaced at several times $2r_0$ in a droplet; say equal to αr_0 . In each rod, the spontaneous annihilation rate for e^-e^+ pairs is approximately given by

$$\tau_s^{-1} \simeq r_0^2 \rho v \quad (5)$$

where r_0 is the classical electron radius, v is the Fermi velocity and ρ the density. The total number of annihilations per second per unit volume is $r_0^2 \rho^2 v$. For the parameters listed above one estimates this to be $10^{38} \text{ s}^{-1} \text{ cm}^{-3}$.

Suppose there is a flux density of e^-e^+ of $\phi \text{ cm}^{-2} \text{ s}^{-1}$ in a given region at the surface of a pulsar. One can roughly estimate the steady-state radius of the droplets, R by equating the annihilations with the introduction of pairs into the droplets assuming the latter occur uniformly over the surface of the droplet. If $R \gg r$, one may conclude that a steady state is possible, and not otherwise. I estimated that $R \simeq 10^{-38} \phi \alpha^2 \text{ cm}$. For the worst case, $\alpha = 1$, one requires $\phi \gg \phi_c \simeq 10^{29} \text{ cm}^2 \text{ s}^{-1}$ to get $R \gg r$.

In one of the more successful models for radiation mechanisms from pulsars^{4,5}, e^-e^+ positron discharges have a crucial role. A flux density of only 10^{24} s^{-1} is allowed in these models from discharges at the 'near gap'⁵. However, an 'outer gap' has been proposed⁶ which generates photons of high enough energy that pair production on magnetic field lines ensues. A flux $\phi \gg \phi_c$ at the surface of the neutron star in the Crab in a region of the polar cap outside the projection of the 'near gap' is quite consistent with this model. Thus the existence of the e^-e^+ droplets depends on this model. Such a flux also accounts for the total number of e^-e^+ annihilations observed by Leventhal *et al.*¹.

Having examined the conditions and characteristics of stimulated annihilation radiation from the droplets, I found that for the length of the droplet greater than 10^{-4} cm , nonlinear amplification of the photons travelling along the length of the drop occurs. The amplification rate is time dependent and the linewidth depends on the amplification rate and can on the average be much less than 3 keV. I also found that the photons thus emitted can escape the magnetosphere around the pulsar with negligible loss. I will publish the details of these calculations later.

I thank P. W. Anderson, E. I. Blount, Andrew Cheng and M. Ruderman for discussion and encouragement. I also wish

to thank M. Leventhal for discussion of his results before publication.

C. M. VARMA

Bell Laboratories,
Murray Hill, New Jersey 07974

Received 22 October 1976; accepted 3 May 1977.

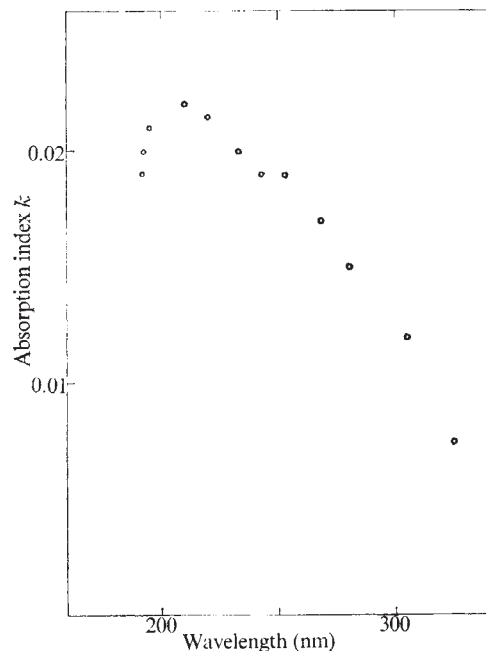
- ¹ Leventhal, M., MacCallum, C. J. & Watts, A. C. *Nature* 266, 696 (1977).
- ² Ruderman, M. *Phys. Rev. Lett.* 27, 1306 (1971).
- ³ Chui, S.-T. *Phys. Rev. B*, 3438 (1974).
- ⁴ Sturrock, P. A. *Astrophys. J.* 164, 529 (1971).
- ⁵ Ruderman, M. A. & Sutherland, P. G. *Astrophys. J.* 196, 51 (1975).
- ⁶ Cheng, A., Ruderman, M. & Sutherland, P. *Astrophys. J.* 203, 209 (1976).

Pre-biotic molecules in Martian dust clouds

THE search for life on Mars has so far proved disappointing¹. Experiments on the Viking package were designed on the presumption that any microbial life on Mars would be endowed with metabolic processes similar to present-day microorganisms on Earth. Analyses of gases released in soil heating experiments² have provided no evidence for significant abundances of highly complex organic molecules. These data are however, limited to only two landing sites and moreover the compositional studies carried out so far reflect properties of only a relatively thin dust layer on the Martian surface. Dust sucked up into the atmosphere from large areas of the planet's surface would have been witnessed in the 1971 storm, representing a much broader sampling of Martian surface material. Here we propose that a broad absorption feature centred on a wavelength close to 2,200 Å, derived from scattering studies of these dust clouds, may be a signature of highly complex organic or prebiotic molecules.

Recently, the possibility that a primitive life form could evolve in the interstellar medium resulting from the formation and destruction of clumps of inorganic grains coated with organic polymers has been discussed³. The identification of the well-known 2,200-Å interstellar absorption band with electronic transitions in a wide class of organic molecules provides tentative support for this

Fig. 1 Absorption index k of Martian dust (1971 dust storms) from Pang and Ajello⁶.



proposal—at any rate for the occurrence of highly complex organic molecules within grains. Also, a similar feature found by Sakata *et al.*⁴ in the organic matter extracted from a carbonaceous chondrite is suggestive of a dormant proto life form in the nature of interstellar grain clumps.

Pang and Ajello⁵ have recently deduced the wavelength dependence of the absorption index k (complex refractive index $m = n - ik$) of Martian dust from an analysis of the scattered light in the 1971 dust clouds observed by Mariner 9. Such clouds are swept up from a large area of the Martian surface and their composition may thus be more representative than that of the exceedingly small sample studied in the Viking experiments. The absorption index deduced from these data is negligible throughout visible wavelengths but increases in the ultraviolet to show a conspicuous hump at a wavelength close to $\lambda = 2,200 \text{ \AA}$ (Fig. 1). The half-width of this band, $\sim 150 \text{ \AA}$, is similar to that of the interstellar extinction band, and also to the absorption band associated with complex organic molecules of the type discussed by Hoyle and Wickramasinghe³. Although a tentative identification of this band with TiO_2 (anastase) grains has been suggested⁵, an association with pre-biotic organic molecules securely lodged within clumps of refractory grains is a distinct possibility which cannot be ruled out by the present data. We note that the strength of the interstellar $\lambda = 2,200 \text{ \AA}$ band is incompatible with absorption by TiO_2 grains (assuming solar abundances), whereas $\sim 10\%$ of interstellar C, N, O in the form of pre-biotic molecules may be shown to provide the observed band strength. Similarly, there would seem to be no compelling geological reason for an over-abundance of Ti on the Martian surface. On the contrary, studies of Martian soil have given an upper limit of 1% (ref. 6) by mass to the titanium content, whereas the titanium composition of dust in the 1971 cloud required to explain the data in Fig. 1 is a few percent⁵. More accurate and extensive spectroscopic studies of Martian dust clouds may well hold vital clues which have a bearing on the question of complex organic molecules and primitive life forms on Mars, as well as their possible connection with a meteoritic and interstellar origin.

H. ABADI
N. C. WICKRAMASINGHE

Department of Applied Mathematics and Astronomy,
University College,
PO Box 78, Cardiff, UK

Received 1 April; accepted 26 April 1977.

¹ Klein, H. P. *et al.* *Science* **194**, 97 (1976).

² Biemann, K. *et al.* *Science* **194**, 72 (1976).

³ Hoyle, F. & Wickramasinghe, N. C. *Nature* **266**, 241 (1977).

⁴ Sakata, A. *et al.* *Nature* **266**, 241 (1977).

⁵ Pang, K. & Ajello, J. M. 1977 *Icarus* **30**, 63.

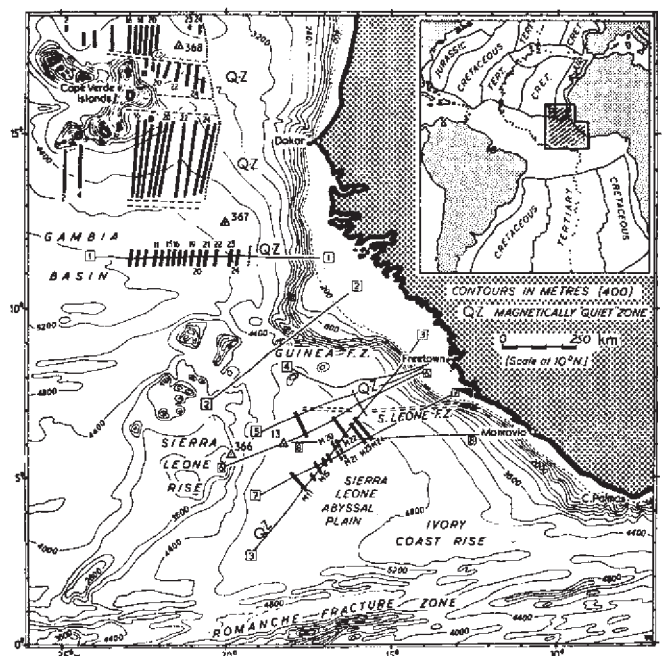
⁶ Toulmin, P. *et al.* *Science* **194**, 81 (1976).

Larson and Hilde⁸. The age of the anomalies provides an important constraint on the timing of the early rifting phase of the Atlantic off Guinea, Sierra Leone and Liberia.

Three magnetic profiles recorded from RRS Shackleton in 1974–75 are presented in Fig. 2. Also included is a published set of anomalies⁶ observed along a WNW–ESE track at 20°N and a corresponding profile computed for a simple spreading model. At 20°N Hayes and Rabinowitz⁶ identified anomalies M2 (Barremian) to M25 (Oxfordian) and have extended this sequence south of the Cape Verde Islands to the vicinity of a fracture zone at 13°N . A magnetically quiet zone occurs to the east which, in its outer portion at least, seems to have resulted from sea-floor spreading during a long period of constant magnetic polarity that ended in the Lower Oxfordian^{5,6} (Fig. 2). Profile 1 lies 110 km south of Glomar Challenger site 367 where sediments of Oxfordian(?)–Kimmeridgian age have been found resting on basaltic basement⁷. On this line, Upper Jurassic anomalies M19–M24 can be identified, together with anomalies of Lower Cretaceous age. The pattern is displaced 160 km in the left-lateral sense from the lineations south-east of the Cape Verde Islands. Whether the displacement takes place entirely at the 13°N fracture zone or along a series of faults cannot be evaluated from present information. A magnetically quiet region occurs on profile 1 but it is significantly narrower than that observed further north (Fig. 1).

Anomalies along tracks between 11°N and 7°N cannot be correlated with those on profile 1, probably because of structural complications associated with the Guinea⁸ and Sierra Leone⁹ fracture zones. Mesozoic lineations are, however, again identified further south. The sequence is

Fig. 1 Mesozoic magnetic lineations in the Eastern Equatorial Atlantic, numbered according to the reversal time-scale of Larson and Hilde⁸. Anomalies north of 13°N are taken from Hayes, Rabinowitz⁶. The inset map is based on crustal ages given by Pitman and others²⁷ and bathymetry is drawn from charts of Uchupi²⁸. Lines 1–6 are tracks of RRS Shackleton. Magnetic anomalies along profiles 7 and 8 have been made available by the Woods Hole Oceanographic Institution and the United States Geological Survey, respectively. Positions 13 and 366–368 are sites drilled by Glomar Challenger. Ages of oldest sediments recovered are: 13 (Senonian; acoustic basement not reached²⁸), 366 (Maestrichtian; acoustic basement not penetrated⁷), 367 (? Oxfordian to Kimmeridgian; acoustic basement penetrated⁷), 368 (Albian; acoustic basement not reached⁷).



Jurassic sea-floor spreading in the eastern Equatorial Atlantic

THE history of sea-floor spreading in the Equatorial Atlantic is poorly known, having been largely inferred from the configuration of fracture zones and the geometry of magnetic lineations in other parts of the Atlantic (Fig. 1). Uncertainties in determining crustal ages at these low latitudes arise partly because of the small amplitudes of the magnetic anomalies and partly because of structural complexities, the separation of South America and Africa involving movements along closely spaced, east-west fracture zones^{1–3}. In this report we describe a series of magnetic anomalies in the eastern Equatorial Atlantic which can be dated using the Vine–Matthews hypothesis⁴ and the time scale of geomagnetic reversals derived by

The development of an oceanic rift in the Sierra Leone Basin during the Jurassic appears to be broadly consistent with the history of adjacent parts of West Africa and offers a plausible explanation for intrusive activity and subsidence near the present continental margin during Jurassic-Cretaceous time. In Liberia and Sierra Leone the edge of the West African craton experienced an episode of igneous activity during the early Jurassic, with the intrusion of tholeiitic dykes parallel to the present continental margin and the emplacement of a deep-seated, basic complex around Freetown¹³⁻¹⁹. The dykes are abundantly developed on land and are known to extend beneath the Liberian shelf, giving rise to linear magnetic anomalies¹⁶. The sharpness of the gravity high and the low amplitude, high frequency magnetic anomalies near km 340 in Fig. 3 probably reflect the continuation of the dyke swarm along the outer part of the Sierra Leone shelf. A tensional stress regime, symptomatic of the initial stages of Atlantic rifting, is thus indicated over a large part of this region during Jurassic time. It is interesting that basic dykes of early Jurassic age have also been reported on the eastern side of the Maranhão Basin in Northern Brazil^{20,21}. On the pre-drift

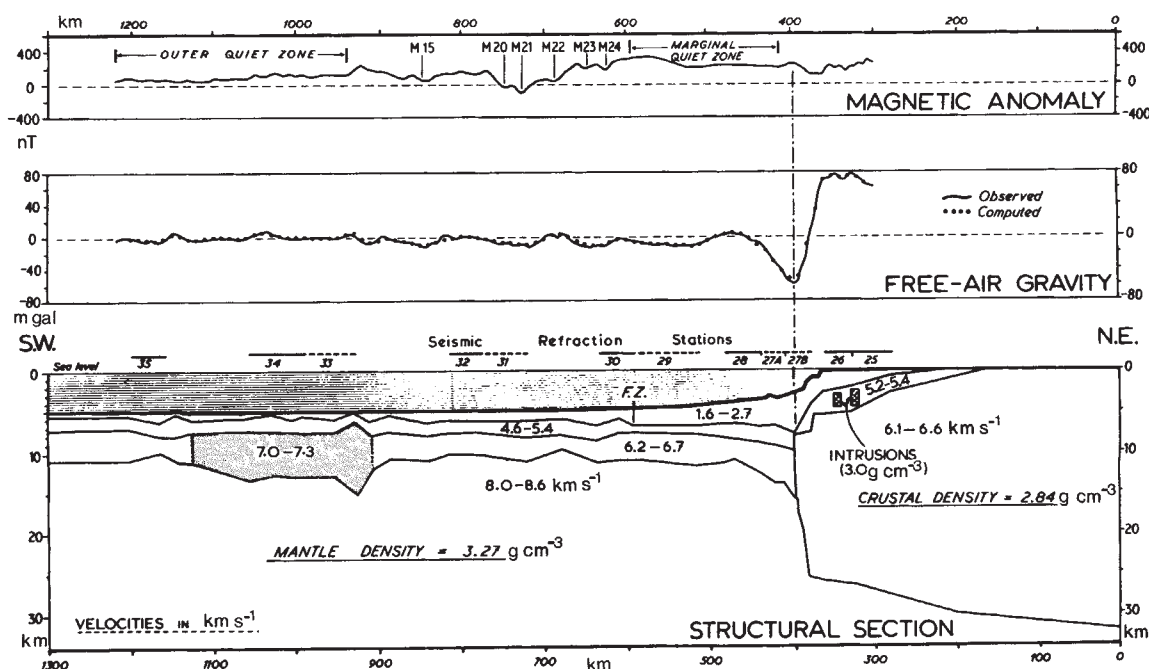


Fig. 3 Crustal structure along profile 3 in Fig. 1 based on published seismic refraction results¹² and the RRS Shackleton gravity profile. Seismic stations indicated by dotted lines are unreversed; the remainder are reversed profiles; F.Z. is the Sierra Leone fracture zone.

continental reconstruction of Bullard and others²² these intrusions form the southern continuation of the Liberian dyke swarm.

In the coastal region of Liberia the Jurassic igneous activity was followed in the Lower Cretaceous by a period of block faulting, subsidence and rapid sedimentation. Four offshore wells near Monrovia reveal a thick section (up to 1,940 m) of dominantly marine Lower Cretaceous sediments resting on basic volcanics, dated as Jurassic at one location and Lower Cretaceous at a second drilling site^{23,24}. Seismic data (ref. 12 and unpublished RRS Shackleton reflection profiles) suggest that more than 2 km of Mesozoic sediments, probably mostly Cretaceous in age, lie beneath the outer shelf off Sierra Leone. The Senegal margin is known to have subsided at least 4 km during the Cretaceous²⁵. However, in this area the first major Mesozoic transgression seems to have occurred before that in western Liberia, as thick marine limestones of Upper Jurassic age have been encountered in boreholes near Dakar. A somewhat later start to rifting off Sierra Leone and Liberia could account for the absence of a continuation of the Triassic-early Jurassic Senegal evaporites²⁶ south of the Guinea fracture zone.

We thank the officers, crew and scientific party aboard RRS Shackleton, the staff of the Research Vessel Base, Barry, and the National Geophysical and Solar-Terrestrial Data Centre, Boulder, for their assistance in this study. Financial support was provided by the Natural Environment Research Council.

E. J. W. JONES
C. C. S. MGBATOGU

Department of Geology,
University College London,
Gower Street, London, UK

Received 24 February; accepted 27 April 1977.

- ¹ Heezen, B. C., Bunce, E. T., Hersey, J. B. & Tharp, M. *Deep-Sea Res.* **11**, 11–33 (1964).
- ² Fail, J. P., Montadert, L., Delteil, J. R., Valery, P., Patriat, P. & Schlich, R. *Earth planet. Sci. Lett.* **7**, 413–419 (1970).
- ³ Le Pichon, X. & Hayes, D. E. *J. geophys. Res.* **76**, 6283–6293 (1971).
- ⁴ Vine, F. J. & Matthews, D. H. *Nature* **199**, 947–949 (1963).

- ⁵ Larson, R. L. & Hilde, T. W. C. *J. geophys. Res.* **80**, 2586–2594 (1975).
- ⁶ Hayes, D. E. & Rabinowitz, P. D. *Earth planet. Sci. Lett.* **28**, 105–115 (1975).
- ⁷ Lancelot, Y., Seibold, E., Cepek, P., Dean, W. E., Eremeev, V., Gardner, J. V., Jansa, L., Johnson, D., Krashennnikov, V., Pflaumann, U., Rankin, J. & Trabant, P. *Geotimes* **20**, 18–21 (1975).
- ⁸ Krause, D. C. *Science* **146**, 57–59 (1964).
- ⁹ McMaster, R. L. & Ashraf, A. *Nature phys. Sci.* **244**, 93–94 (1973).
- ¹⁰ Poehls, K. A., Luyendyk, B. P. & Heirtzler, J. R. *J. geophys. Res.* **78**, 6985–6997 (1973).
- ¹¹ Barrett, D. L. & Keen, C. E. *J. geophys. Res.* **81**, 4875–4884 (1976).
- ¹² Sheridan, R. E., Houtz, R. E., Drake, C. L. & Ewing, M. J. *J. geophys. Res.* **74**, 2512–2530 (1969).
- ¹³ Talwani, M. & Eldholm, O. *Nature* **241**, 325–330 (1973).
- ¹⁴ Larson, R. L. & Ladd, J. W. *Nature* **246**, 209–212 (1973).
- ¹⁵ Behrendt, J. C. & Woterson, C. S. *Geol. Soc. Am. Bull.* **81**, 3563–3574 (1970).
- ¹⁶ Behrendt, J. C. & Woterson, C. S. *U.S.A. Geol. Surv. Prof. Paper* **810** (U.S.A. Government Printing Office, Washington, 1974).
- ¹⁷ Wells, M. K. *Overseas Geol. Miner. Resources Bull. Suppl.* No. 4 (Overseas Geol. Surveys, London, 1962).
- ¹⁸ Baker, C. O. & Bott, M. H. P. *Overseas Geol. Miner. Resources* **8**, 260–278 (Overseas Geol. Surveys, London, 1961).
- ¹⁹ Briden, J. C., Henthorn, D. I. & Rex, D. C. *Earth planet. Sci. Lett.* **12**, 385–391 (1971).
- ²⁰ Kegel, W. *Geol. Min. Div. Bol., Brasil* No. 227 (1965).
- ²¹ May, P. R. *Geol. Soc. Am. Bull.* **82**, 1285–1292 (1971).
- ²² Bullard, E. C., Everett, J. E. & Smith, A. G. *Phil. Trans. R. Soc. A* **258**, 41–51 (1965).
- ²³ Schlee, J., Behrendt, J. C. & Robb, R. M. *Am. Assoc. pet. Geol. Bull.* **58**, 708–728 (1974).
- ²⁴ Cortesini, A. & Minner, J. R. *Am. Assoc. pet. Geol. Bull.* **56**, 1749–1792 (1972).
- ²⁵ De Spengler, A., Castelain, J., Cauvin, J. & Leroy, M. in *Bassins Sédimentaires du Littoral Africain* (ed. Reyre, D.) 80–94 (Assoc. Serv. Géol. Afr., Paris, 1966).
- ²⁶ Templeton, R. S. M. *Inst. geol. Sci. Rep.* **70**/1647–60 (1971).
- ²⁷ Pitman, W. C., Larson, R. L. & Herron, E. M. *The Age of the Ocean Basins* (Map published by Geological Society of America, Boulder, 1974).
- ²⁸ Uchupi, E. *Bathymetric Atlas of the Atlantic, Caribbean and Gulf of Mexico* (Woods Hole Oceanographic Inst. Ref. 71–72, 1971).
- ²⁹ Maxwell, A. E., von Herzen, R. P., Andrews, J. E., Boyce, R. E., Milow, E. D., Hsu, K. J., Percival, S. F. & Saito, T. *Init. Rep. deep sea Drill. Proj.* **3**, 27–45 (U.S.A. 1970).

Late Quaternary lake levels in southern Afar and the adjacent Ethiopian Rift

A PERENNIAL problem in using evidence of changing lake levels and river activity to reconstruct Quaternary environments in East Africa stems from our frequent inability to distinguish between effects produced by volcanic and tectonic activity and those related to climatic change. We report here evidence for roughly synchronous changes in lake levels in the volcanically and tectonically active regions of southern Afar and the Ethiopian Rift, which parallel those reported from the Sahel¹, the Nile valley², Kenya³ and Saudi Arabia⁴. Such synchronous changes in lake levels,

allied to independent evidence of moister conditions during phases of high lake levels, are best explained through regional fluctuations in climate.

Lake Besaka is situated at the junction of the Ethiopian Rift and the Afar triangle (Fig. 1), and is bounded to the west and east by pre-Holocene fault scarps which run NNE-SSW, parallel to the regional trend of faults in the Wonji Fault Belt. The northern margin of the lake consists of very fresh, unvegetated aa lava which earlier observers^{5,6} attributed to an eruption in the 1820s. Morphologically fresh cinder cones mark the present southern edge of the lake (Fig. 2). Late Holocene to modern tensional fissures at least 30 m deep and up to 4 m wide extend north through the welded tuffs on the southern flanks of Fantale volcano.

The lithostratigraphic sequence above and below the western fault scarp at Lake Besaka (Fig. 3) shows that the lake attained a level of about 10 m above the January 1974 datum level towards 11,000–15,000 BP (Table 1), shortly after a major eruption of olive-green pumice sand and gravel. Above the scarp the pumice is an airfall deposit that covers a Late Stone Age site with nearly a metre of coarse sand and sporadic gravel. Overlying the green sand is a second Late Stone Age occupation layer with abundant obsidian implements, faunal remains and human burials. Quantitative analysis by X-ray fluorescence of the strontium, yttrium and zirconium content of the green sand above and below the scarp and within the marginal fissure site E3 W (Fig. 3) shows that the trace-element content is identical at all sites, as are the refractive indices of the glass and the mineral composition of the sand. The only differences lie in the sedimentary structures and upward coarsening evident in the green sand below the scarp, which reflect deposition within or resorting by the lake.

Fig. 1 Location map of sampled sites.

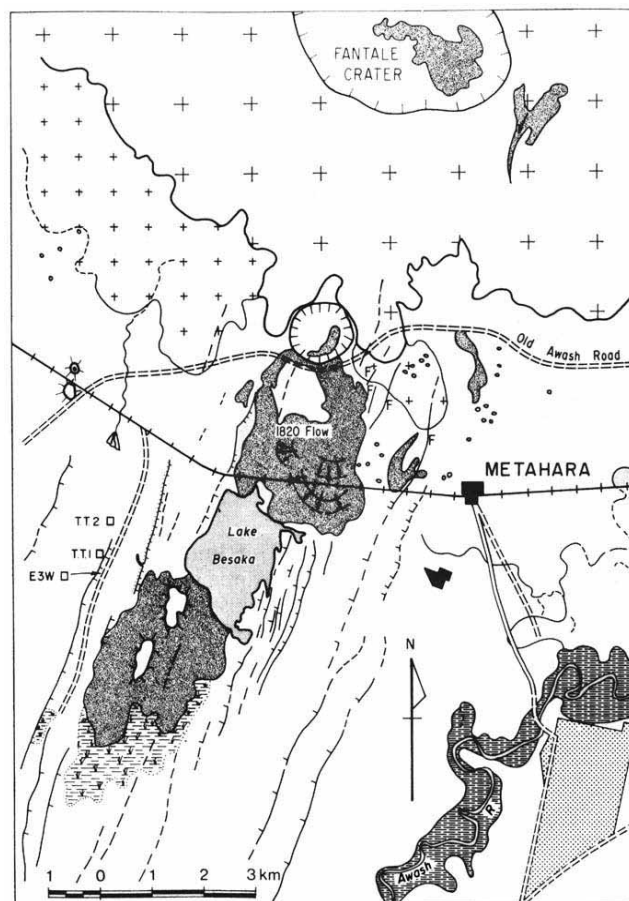
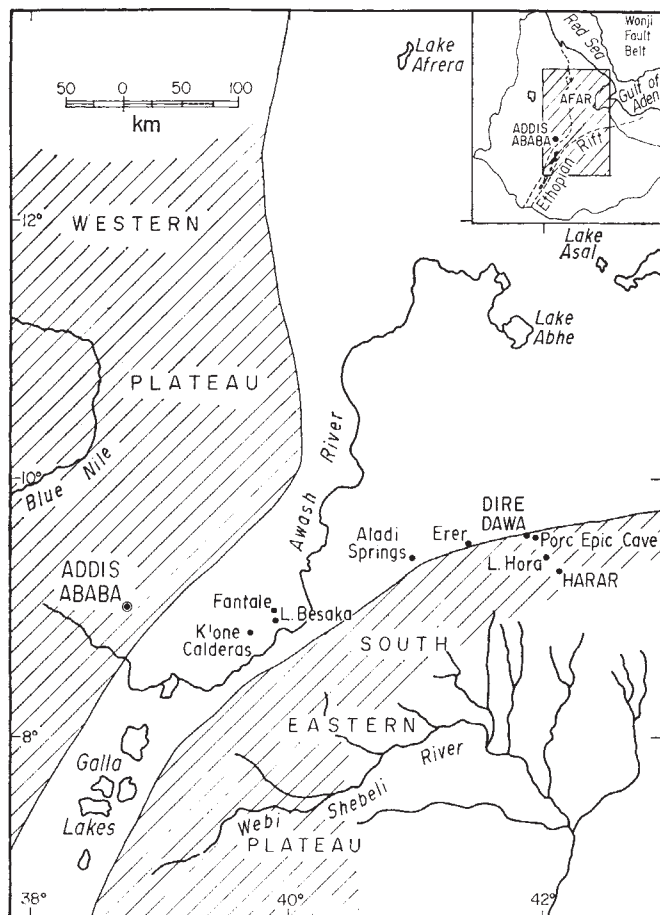


Fig. 2 Geomorphic map of Lake Besaka. The sketch map is based on air photographs taken in 1957.

The lake level was high ($\geq +10$ m) for an unknown interval during the late Pleistocene before the deposition of the green sand, and fell sufficiently for Late Stone Age man to move out across the former lake floor down to and perhaps lower than an elevation of $+4.5$ m.

A fall in level following deposition of the 11,000–11,500 BP shell-bearing diatomites above the green sand is evidenced by dark organic clays that locally contain Late Stone Age flakes. The lake rose again during the Holocene to reach the base of the fault scarp at $+10$ m, and a second layer of diatomite accumulated above the dark clay towards or shortly after 9,500 BP (Table 1). Before attempting to interpret the changing late Quaternary levels of Lake Besaka, it is pertinent to review evidence from the area as a whole (Fig. 1; Table 1).

K'one caldera complex⁷ lies in the Ethiopian Rift some 30 km WSW of Lake Besaka. Recent gully erosion has revealed a horizontal sequence of alternating dark grey clays and brown tuffaceous loams above the welded tuff and

Table 1 Radiocarbon ages from south-central Ethiopia

Specimen no.	^{14}C age (b.p.)	Site	Material	Comments
I-8322	$14,670 \pm 200$	K'one	Ostrich eggshell	Just below upper dark clay
I-8325	$11,430 \pm 380$	L. Besaka	Shells	<i>Melanoides</i> at 4.2 m
I-7969	$11,200 \pm 160$	L. Besaka	Shells	<i>Melanoides</i> at 6.5 m
I-7970	$11,070 \pm 160$	Aladi Springs	Shells	<i>Melanoides</i> in LSA tufa
SUA-461	$9,410 \pm 140$	L. Besaka	Shells	<i>Melanoides</i> at base of dark clay
I-8326	$6,890 \pm 115$	L. Hora	Shells	Mixed littoral assemblage
SUA-462	$6,810 \pm 120$	K'one	Carbonate*	Base of upper dark clay
I-8324	$6,670 \pm 110$	Erer	Shells	<i>Melanoides</i> in tufa
I-7971	$5,700 \pm 110$	Porc Epic	Charcoal*	In calcareous MSA breccia
I-8330	$4,785 \pm 120$	L. Besaka	Charcoal	Near top of LSA site
SUA-459	$4,750 \pm 160$	L. Besaka	Carbonate*†	LSA soil below green pumice
SUA-460	$4,220 \pm 120$	L. Besaka	Humic clay	Above shelly diatomite

*Younger than deposit in which it occurs.

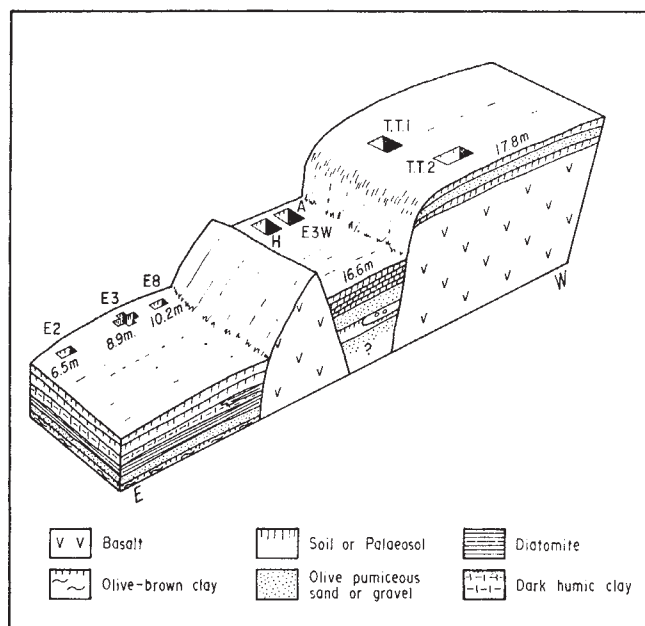
†Contaminated by much younger carbonate. Mean of two ages: inner sample: $4,490 \pm 115$; outer sample: $5,010 \pm 110$.

thin overlying pumice airfall deposit lining the floor of a degraded caldera. The upper of the three dark clays contains Late Stone Age artefacts, and the transition from Middle to Late Stone Age assemblages at the base of this clay is dated at $14,670 \pm 200$ BP (Table 1). The regular alternation of horizontal clays and loams from Middle to Late Stone Age time, the moisture-tolerant terrestrial mollusc fauna of the middle dark clay, and the present-day lack of standing water in this locality all imply deposition of the clays in moister conditions than those prevailing there today. If so, then the final interval of clay deposition after 14,670 BP was a relatively humid one, and it is significant that further south in the Galla Lakes region of the Ethiopian Rift lake levels rose at or soon after 14,400 BP (ref. 8).

At Aladi Springs, about 150 km north-east of Lake Besaka, *Melanoides tuberculata* shells from a tufa containing Late Stone Age implements have yielded an age of $11,070 \pm 160$ BP, which is similar to that for high lake levels at sites in central Afar⁹, the Awash valley¹⁰ and the Ethiopian Rift⁸. We conclude that the 11,000–11,500 BP high level recorded at Lake Besaka was not a solitary local event but was triggered by a climatic oscillation of regional importance.

Two southern Afar sites some 120 km apart show signs

Fig. 3 Block diagram of late Quaternary deposits west of Lake Besaka (not to scale).



of wetter conditions towards 7,000 BP. Lake Hora near Harar was at least 1 m deeper than now at $6,890 \pm 115$ BP; and a shelly tufa was deposited under permanent water on the flood-plain of a now seasonal stream between Aladi Springs and Erer at $6,670 \pm 110$ BP. Lakes Abhe, Afrera and Asal in central Afar⁹ were also very high at this time, and it may be no coincidence that calcium carbonate was being precipitated at the base of the upper dark clay at K'one some $6,810 \pm 120$ yr BP.

A Late Stone Age site above the western fault scarp at Lake Besaka is dated at $4,785 \pm 120$ BP for undisturbed occupation levels near the surface. Calcium carbonate nodules within the pre-Holocene lake clay beneath the green sand below the scarp have a mean age of $4,750 \pm 160$ BP, suggesting mobilisation of younger carbonate at about this time. A single anomalously young date of $5,700 \pm 110$ BP was obtained on charcoal collected from a late Pleistocene cave-breccia containing Middle Stone Age obsidian points at Porc Epic limestone cave near Dire Dawa.

Shell and carbonate are far from ideal for radiocarbon dating, so that a search for more suitable samples is now under way at Lake Besaka, and geochemical assays of the presently dated samples are in progress. Nevertheless, and despite the equivocal significance of certain of these dates (notably I-7971 and SUA-458) it is worth noting that the central Afar lakes⁹ were high from around 10,000–11,000 BP to around 8,500 BP and again from around 7,500 BP to around 4,000 BP. After an early rise in the level of the lakes of the Ethiopian Rift⁸ towards 14,400 BP, lake levels were very high just after 9,500 BP and again towards 6,500 BP. The present evidence from Lake Besaka, K'one, Aladi Springs, Erer and Lake Hora is entirely consistent with other data from outside Ethiopia^{1–4}, as well as with independent evidence from the Ethiopian Rift and Afar^{8–10}, and indicates wetter-than-present conditions after around 14,500 BP, with notably moist climates towards 11,000–11,500 BP, 9,500 BP, 6,500–7,000 BP and 4,000–5,000 BP. The early Holocene climates in south-central Ethiopia thus show signs of four wet pulses followed by progressive desiccation after 4,000 BP. The late Holocene in Ethiopia was a time when increasing burning and clearing of the land for cultivation in the uplands, and grazing by herds of domesticated cattle, sheep and goats in the drier lowlands would have accentuated the biological pressure exerted by falling water-tables and reduced soil-water content on a vegetation adapted to the moister climates of the early Holocene.

This was part of a continuing investigation of the palaeo-anthropology of south-central Ethiopia, directed by Professor J. Desmond Clark, to whom we extend our thanks. We thank NSF for finance, Macquarie University for leave, Dr W. H. Morton for assistance, Drs D. Brown and

B. Verdcourt for identifying the mollusca, and our Ethiopian colleagues for support.

M. A. J. WILLIAMS
PAUL M. BISHOP
FRANCES M. DAKIN

School of Earth Sciences,
Macquarie University,
North Ryde, NSW 2113,
Australia

R. GILLESPIE

Sydney University Radiocarbon Laboratory,
Department of Physical Chemistry,
University of Sydney, NSW 2006,
Australia

Received 13 December 1976; accepted 25 March 1977.

- ¹ Street, F. A. & Grove, A. T. *Nature* **261**, 385–390 (1976).
- ² Williams, M. A. J. & Adamson, D. A. *Geogr. J.* **139**, 498–508 (1973).
- ³ Butzer, K. W., Isaac, G. L., Richardson, J. L. & Washbourn-Kamau, C. *Science* **175**, 1069–1076 (1972).
- ⁴ McClure, H. A. *Nature* **263**, 755–756 (1976).
- ⁵ Harris, W. C. *The Highlands of Ethiopia* 3 (Longman, Brown, Green and Longman, London, 1844).
- ⁶ Buxton, D. R. *Travels in Ethiopia* (London, 1949).
- ⁷ Cole, J. W. *Bull. Volc.* **33**, 566–578 (1969).
- ⁸ Grove, A. T., Street, F. A. & Goudie, A. S. *Geogr. J.* **141**, 177–202 (1975).
- ⁹ Gasse, F. thesis, Paris (1975).
- ¹⁰ Taieb, M. thesis, Paris (1974).

The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment

FATTY acids continue to provide insights into the origins and reactions of organic matter in Recent sediments^{1–4}. As part of a wider baseline study^{1,2} we have isolated the fatty acids from a typical temperate intertidal zone sediment located at the south-west of Corner Inlet, Victoria¹. The acid concentration in this core section is very low (13.4 µg per g sediment) and in contrast to most sediments the mono-unsaturated acids predominate over the saturated acids (Table 1). There are also significant quantities of poly-unsaturated acids (Table 2) which have rarely been identified in sediments^{5,6} and there has been no detailed study of the isomers present. The carbon number pattern taken together with the degree of unsaturation and the isomer positions are used in this report to confirm a marine diatom input to the sediment distinguishable from that of other micro-organisms.

The sediment core (13–19 cm deep) was collected 6 m above the low water mark from beneath a bed of *Zostera marina* which stabilised the sediment. Terrestrial plant input is minimal. The sediment was sieved to remove any larger organisms and the total extract was obtained by

KOH-methanol extraction¹. Rigorous precautions were taken to exclude contamination and blank runs were performed. The carboxylic acids were obtained by partitioning the acidified extract into heptane-diethyl ether mixtures. The methyl esters (BF₃-MeOH) were purified on silica gel and 5% AgNO₃ silica gel plates. Small quantities of long chain α,ω -dicarboxylic acids and hydroxylated acids were also present¹.

Gas-liquid chromatography utilised glass SCOT SE30 (60 m × 0.5 mm ID; S.G.E. Australia), and stainless steel capillary BDS (46.7 m × 0.25 mm ID; Perkin Elmer) columns. Compound identification was by co-injection and relative retention time measurements. Hydrogenation in methanol over Adams catalyst confirmed the presence of unsaturated acids.

The sedimentary fatty acid concentrations are high in 16:0 (18%), 16:1 ω 7, 20:4 ω 6 and 20:5 ω 3 and low in C₁₈ components (Table 2). This pattern closely resembles that of many species of marine diatoms^{7–9} and is quite unlike most other algal species^{10,11}. A diatom input is further suggested by the positional isomers of the polyunsaturated acids present.

The three 16:2 isomers commonly found in marine phytoplankton are ω 4, ω 6 and ω 7 but only the isomers (ω 4 and ω 7) commonly found in marine diatoms^{9,10} are found in the sediment. Jamieson and Reid¹¹ reported only the ω 4 and ω 6 isomers in their extensive study of macroscopic Rhodophyta, Phaeophyta and Chlorophyta species. Similarly, of the three 16:3 isomers present, only the ω 4 is in significant quantity. This is the only isomer reported by Ackman and Tocher¹⁰ for four different diatom species. The virtual absence of the ω 3 isomer indicates that Chlorophyceae in particular can make only a small contribution to the sediment.

The low levels of C18 unsaturates also suggest a minor contribution from higher plants or green algae. Of the two 18:3 isomers identified, it is the less abundant ω 3 isomer which is common to plants and most algae^{10,11}. The tetra- and penta-unsaturated components isolated, while typical of marine diatoms^{7–10}, are also found in varying amounts in other algae¹¹. The complete absence of 22:6 reflects the small contribution from higher marine organisms.

The abundance of these highly unsaturated molecules in the sediment is unusual as it is generally believed that they are rapidly degraded in a sedimentary environment⁴. Cranwell¹² was unable to detect polyunsaturated acids in a lake sediment despite the fact that the presumed major source of the sediment fatty acids were planktonic organisms which contain large quantities of these acids.

This rapid degradation reflects the aerobic conditions to which the unsaturated acids presumably were exposed¹². In our sediment most of the unsaturated acids will be deposited directly into the sediment when the diatoms die, and hence the acids will largely escape the aerobic conditions at the surface. Preservation is then aided by the anaerobic conditions below the surface ($E_H \approx -120$ mV at the studied depth).

Bacteria are known to contribute branched (iso, anteiso and cyclopropyl) acids to sediments^{2,3} but their contribution to unsaturated acids has been less certain². Our sediment contains a wide range of mono-unsaturated acids and particularly significant levels of vaccenic acid (18:1 ω 7). Unlike plants and most algae^{9,10} bacteria usually have vaccenic acid and not oleic acid as the major 18:1 isomer¹³. We believe that bacteria contribute most of the 18:1 ω 7 and a significant proportion of the other mono-unsaturated acids in the sediment. Recent results (to be published) from bacteria isolated from the sediment support this view. Probably the branched unsaturated acids isolated are also derived from bacteria.

Van Vleet and Quinn¹⁴ have recently examined the 16:1 and 18:1 isomers in an estuarine sediment and found 8%

Table 1 Concentrations of different acid classes

Type	Number of double bonds	Carbon number (range)	Concentration (µg acid per 100 g sediment)
Straight chain	0	10–30	421.0
Branched*	0	11–19	91.0
Straight chain	1	12–18	517.0
cis			
Straight chain	1	12–18	13.3
trans†			
Branched	1	15–18	2.8
cis			
Straight chain	2	16–18	50.2
cis			
	3	16–20	49.3
	4	18–22	115.0
	5	20–22	82.9

* Mainly iso and anteiso, some cyclopropyl 17:0, 19:0, trace 10-methyl 16:0. † Only t-16:1, t-18:1 positively identified and quantitated.

Table 2 Concentration of unsaturated acids

Acid	Percentage of total acids*	Concentration ($\mu\text{g per 100g sediment}$)	Acid	Percentage of total acids	Concentration ($\mu\text{g per 100 g sediment}$)
12: 1†	0.12	1.55	17: 2†	0.28	3.64
14: 1ω7	0.24	3.26	18: 2ω6	1.34	18.0
14: 1ω5	0.12	1.54	16: 3ω4§	1.28	17.1
15: 1ω8	0.30	3.97	18: 3ω3	0.45	6.03
15: 1ω6	0.42	5.56	18: 3ω6	0.86	11.6
iso 15: 1	0.11	1.51	20: 3ω6	0.43	5.76
anteiso 15: 1	0.04	0.48	18: 4ω3	0.76	10.1
16: 1ω7	29.2	390	20: 4 ω6	8.39	112
16: 1ω5	0.55	7.36	22: 4ω6	0.25	3.37
iso 16: 1‡	0.06	0.83	20: 5ω3	7.30	97.8
17: 1ω8	2.14	28.7	22: 5ω3	0.12	1.66
17: 1ω6	0.45	6.02			
iso 17: 1‡	0.25	3.29	t16: 1ω7	0.27	3.56
anteiso 17: 1	0.09	1.17	t16: 1ω13	0.47	6.32
18: 1ω9	1.88	25.2	t18: 1ω7	0.14	1.89
18: 1ω7	3.30	44.2	t18: 1ω9	0.11	1.51
16: 2ω4	1.26	16.9			
16: 2ω7	0.87	11.6			

* Data quoted to two decimals is only used to indicate the ratio of minor components. Actual accuracy is about 15 % and the values are corrected for the slight loss of unsaturated acids on the capillary columns. † Two unidentified isomers. ‡ Mainly Δ⁹. § Trace ω3 and ω6 isomers.

in each case were *trans*. In our sediment the percentage of the *cis* isomer is larger (99% for 16: 1, 95% for 18: 1) and the proportion of *cis* and *trans* for each positional 16: 1 and 18: 1 isomer varies significantly. The range of *trans* acids in the sediment seems to parallel the range of the corresponding *cis* isomers but the very low concentrations encountered here have prevented us from obtaining unequivocal identifications. These acids and the change of the *cis-trans* ratio with depth are under study. The other *trans* isomer isolated, t16: 1ω13, is probably of biological origin. This acid has not previously been reported from a sediment, despite its ubiquity in photosynthetic organisms where it is found localised in the chloroplast phosphatidyl glycerol fraction¹⁵. This acid may prove to be a good marker for a contribution from photosynthetic organisms.

This report shows that a detailed study of the gross fatty acid patterns can be combined with the isomeric forms of these acids to be of value to the environmentalist in relating isolates to probable origin. In our case diatoms and marine bacteria are strongly implicated as the major contributors of organic carbon to this typical, temperate intertidal zone sediment.

The authors thank the ARGC for financial support, and G. J. Perry and F. T. Gillan for valuable discussions. J. K. Volkman acknowledges a C.P.G. Award.

J. K. VOLKMAN
R. B. JOHNS

Department of Organic Chemistry,
University of Melbourne,
Parkville, Victoria 3052, Australia

Received 31 January; accepted 30 March 1977.

- Johns, R. B. & Onder, O. M. *Geochim. Cosmochim. Acta* 39, 129-136 (1975).
- Johns, R. B., Perry, G. J. & Jackson, K. S. *Estuarine and Coastal Mar. Sci.* 5, (1977) (in the press).
- Parker, P. L. in *Organic Geochemistry* (eds Eglinton, G. & Murphy, M. T. J.) (Springer, Berlin, 1969).
- Farrington, J. W. & Quinn, J. G. *Nature phys. Sci.* 230, 67-69 (1971).
- Peterson, D. H. thesis, Univ. Washington (1967).
- Poltz, V. J. *Arch. Hydrobiol.* 4, 315-399 (1972).
- Opute, F. I. *J. exp. Bot.* 25, 823-825 (1974).
- Kates, M. & Volcani, B. E. *Biochim. biophys. Acta*, 116, 264-278 (1966).
- De Mort, C. I. Lowry, R. Tinsley, I. & Phinney, H. K. *J. Phycol.* 8, 211-216 (1972).
- Ackman, R. G., Tocher, C. S. & McLachlan, J. J. *Fish. Res. Bd Can.* 25, 1603-1620 (1968).
- Jamieson, G. R. & Reid, E. H. *Phytochem.* 11, 1423-1432, (1972).
- Cranwell, P. A. *Freshwater Biol.* 6, 41-48 (1976).
- Auran, T. B. & Schmidt, E. L. *J. Bacteriol.* 109, 450-451 (1972).
- Van Vleet, E. S. & Quinn, J. G. *Nature* 262, 126-128 (1976).
- Harwood, J. L. & James, A. T. *Eur. J. Biochem.* 50, 325-334 (1975).

Are monkeys logical?

THE monkey's status as a thinker has never been high; yet laboratory investigations testify, nevertheless, to the ability of many species of monkey to learn complex tasks, if not to reason. On this latter point, however, hard evidence is significantly lacking. One reason for this is that it is difficult to devise tests which are both meaningful to non-verbal subjects yet satisfy the stringent requirements of a formal reasoning test such as one adapted from Burt¹ which first gives the subject the following information: "Edith is fairer than Suzanne", "Edith is darker than Lili", and then requires solution of the question, "which is the darkest, Edith, Suzanne or Lili?". Bryant and Trabasso² have devised a simplified method of giving such tests to very young children, and we have adapted this into a non-verbal one for use with monkeys.

In Bryant and Trabasso's study, children were first trained to label five rods presented in four subsets as big or small according to the colour and the subsets in which they were embedded. As displayed, however, no size differences were apparent. Thus, for example, a yellow rod (A) was taught as 'taller than' a red one (B), a red one as 'taller than' a blue one (C), a blue rod was learnt as 'taller than' a green one (D), and a green one as 'taller than' a white one (E). On subsequent tests, children were asked to judge the relative sizes of non-adjacent members of the set, for example red (B) against green (D).

Eight adult squirrel monkeys were tested in an apparatus (a Visconsin General Testing Apparatus) which allowed the monkey to view on any one trial, a tray bearing two cylindrical tin containers of equal size but differing in colour and weight. The experimental design is depicted in Table 1. Each monkey was confronted with four pairs of colour discriminations, learnt serially. For half the subjects the rewarded stimulus was heavier than the non-rewarded one; for the other half it was the lighter one. Weight differences were used to emphasise stimulus differences in the course of preliminary training³. Only two weight values were used throughout the experiment, however; the 'heavy' tin in any pair was filled with lead shot, the 'light' one was empty. Thus, no specific weight could be uniquely identified with stimuli B, C and D.

Monkeys were assigned to different colour pairings and the tray colour was also varied from monkey to monkey in an attempt to eliminate fortuitous colour preferences which might affect the crucial test choices. Each pair was

Table 1 Scheme of training paradigm

Series identification		A	B	B	C	C	D	D	E
Pair		1		2		3		4	
Weight	4 subjects	Light	Heavy	Light	Heavy	Light	Heavy	Light	Heavy
Colour (for example)	4 subjects	Yellow	Blue	Blue	Green	Green	Red	Red	White

learnt in serial order from AB to DE to a criterion of 18/20 trials correct for each pair. Choice of one of the tins was rewarded with a peanut located in one of two foodwells directly underneath the stimuli. To indicate choice and secure the reward, the monkey had to manually displace the appropriate tin from its position over the foodwell. After an error, however, the monkey received no reward; the tray was withdrawn in full view of the subject for 5 s, and a screen was then lowered to permit the experimenter to replace the stimulus unseen and restart testing. The respective positions of the stimuli varied from the left to the right of the tray according to a random sequence.

When subjects had learnt all four pairs they were given a second run through all problems in the same order to the same performance criterion. Several subsequent runs then followed in which the number of training trials given per problem was progressively reduced. Finally, five runs were given of one trial per problem in a random order of presentation. A maximum of 50 trials per day was administered. When each monkey had progressed through all stages of the procedure outlined above, it was given a series of 10 critical trials on the B against D comparison (see Table 1). 'Critical' tests, however, were not administered in any session until the animal first recorded a performance of 22/24 correct in the course of 24 successive encounters of training pairs in a random sequence; not more than two transitive tests were administered per session. All choices were rewarded irrespective of bias during the testing phase. When the B against D tests were complete, subjects were given further tests in similar conditions with the AC, AD, AE, CE and BE combinations in counterbalanced order of presentation (these are regarded as less critical as they include stimuli A and E which can be identified as having been invariably rewarded (E) or invariably non-rewarded (A)). All but one monkey learnt the series; the eighth was erratic and did not meet our stringent training criteria sufficiently often to warrant subsequent testing.

In Table 2 the monkey results are compared with the child data obtained by Bryant and Trabasso for 4-yr-old children. These data clearly show a choice profile consistent with the notion that monkey choices are transitive and accord in virtually every detail with the data reported for children.

Are we to assume, therefore, that monkeys solve such problems by means of deductive inference? Certainly the evidence is consistent with the idea that in order to solve such problems, subjects must coordinate two vital pieces of information—for example that C is heavier than B, and that D is heavier than C. There are alternative ways in

which the problem might be solved, however. Among these⁴, the one which is perhaps the most radically at odds with the coordination model² assumes that transitive choices result from single binary decision making.

A set BCD, for example, affords three subsets, BC, CD and BD. If a decision is based exclusively on the interrogation of any one subset, and if we assume (in the absence of information to the contrary) that each subset is interrogated equally often, then it is possible to compute the choice proportions to each of the constituent members of the triad BCD on a given trial. If, therefore, the probability of selecting any one of the subsets as a basis for choice is 0.33 (approximately) we can thus predict an overall choice proportion of 0.33 in the case of C (assuming absolute preference for C over B). D has two reference subsets, CD and BD. In the case of subset CD (making the same assumptions as above) the choice proportion predicted for D will be approximately 33%. In the case of subset BD, however, we need assume no consistent preference for either B or D. On the 33% of all occasions on which these comparisons are made, the subject may select either one equally often. In this case, the overall probability of choosing D would rise by a further 16% (with roughly 16% of all choices going to B). Thus we might predict the approximate choice proportions for all three stimuli in the set BCD as follows: B = 0.17, C = 0.33 and D = 0.50.

In a two-choice situation, of course, where B is presented in conjunction with D, C would have to be 'inferred' as a referent (an assumption in common with the coordination model, some theories of perception⁵, and of transitivity⁶ and a reasonable one given the duplicate relationship of C with B and D). In this instance the choice proportions attributable to C when actually present will now add to the overall proportions for D (as half of them will rule out responses to B, the other half will confirm D directly). Thus the probability values for BD in a two-choice situation will now be: B = 0.17, D = 0.83.

In the case of comparisons involving an endpoint value, however, (either A or E), fewer subsets will be at chance levels of choice (taking as a total population all 10 triadic permutations of the 5 term series A, B, C, D and E); thus the projection for the average transitive choice proportion is 0.98. Such predictions seem a remarkably close fit to data already obtained in various experiments reported to date (Table 3).

Apart from fitting predictions to existing data, however, to provide an alternative account to the coordination model, there is an empirical test of the relative strengths of these opposed accounts which is made possible if three

Table 2 Transitive choice data for squirrel monkeys compared with the profile reported by Bryant and Trabasso² for 4-yr-old children.

	Monkeys				4-yr-old children (B+T) (Experiment 1)			
	B	C	D	E	B	C	D	E
A	0.98	1.00	1.00	1.00	A	0.96	0.96	0.98
B	—	0.93	0.90	0.76	B	—	0.92	0.92
C	—	—	0.89	0.87	C	—	—	0.94
D	—	—	—	0.97	D	—	—	0.91

All choices reported for monkeys are significant on a binomial test ($P < 0.001$). Note especially the B against D comparisons which cannot depend directly on 'end-anchoring' effects.

Table 3 Averaged transitive choice data taken from three separate investigations (choice proportions)

Investigation	Choice proportions to pairs with end points	Proportions to the 'critical' pair (BD)	Overall transitive choice proportion
Bryant and Trabasso ² experiments 1 and 2 (5 groups)	0.96 (0.98)	0.85 (0.83)	0.94 (0.96)
de Boysson-Bardies and O'Regan ⁷ experiments 1 and 2 (2 groups)	0.91 (0.98)	0.82 (0.83)	0.90 (0.96)
This work (1 group)	0.93 (0.98)	0.90 (0.83)	0.92 (0.96)
Average (all investigations)	0.95 (0.98)	0.85 (0.83)	0.93 (0.96)

Values in parentheses are predicted values from a binary choice model (basis of computation outlined in Table 4).

(or more) stimuli, for example B+C+D, are actually presented to the subject for choice following establishment of strong preferences within subsets BC and CD. On the coordination model it is not readily apparent why the actual presentation of the three relevant terms would do other than emphasise further the choice bias in favour of D obtained in the course of two-choice transitivity tests (for example BD). On the binary sampling model, however, a 'reduced' transitive effect is demanded whereas no such predictions are afforded by the coordination theory. We report below a direct test of these positions.

The seven remaining monkeys were presented with 10 triplets derived from all possible triadic combinations of the five stimuli in the original set (A, B, C, D and E). Each session began with 25 trials involving the original training pairs presented in random order. If not more than two errors were recorded, monkeys were then confronted with a three-choice situation where, for another 25 trials per session, each of the 10 triplets was presented in a counterbalanced order. The position of each stimulus on the tray was also counterbalanced. The stimuli were either all 'heavy' or 'light' in accordance with the previous training history of the animal. All choices were rewarded, and the experiment was terminated when subjects made 10 separate choices to each of the 10 triplets presented for test.

Table 4 Profile of choice following triadic presentations

Triads	Choice projection*			Obtained		
ABC	00	33	67	00	31	69
BCD	17	33	50	03	36	61
BDE	17	17	67	16	24	60
CDE	00	33	67	11	24	65
BCE	00	33	67	06	28	66
ABD	00	50	50	00	44	56
ACD	00	33	67	00	30	70
ADE	00	33	67	01	21	78
ABE	00	33	67	00	30	70
ACE	00	33	67	00	26	74
Average distribution	0.03	0.33	0.64	0.04	0.29	0.67

The figures in the left-hand column are predictions based on the assumptions that the subsets are sampled equally often and that preferences are absolute within those subsets presented during original training.

*Basis for predictions in Table 3.

Table 4 shows the overall choice profile in these conditions, and shows how the response distributions for triads are well predicted from a binary (statistical) decision model. By contrast, a coordination model² does not readily lend itself to any quantifiable prediction of a reduced transitive effect. On the contrary, with the relevant choice items in the immediate perceptual field, the 'transitive' choices should, if anything, be all the more pronounced.

Whatever the case for, or against, deductive reasoning which may be fashioned from results such as those reported here, it is clear that some kind of 'inference' necessary to produce the appropriate 'inferred' set or absent referent is used by monkey in tests of transitivity such as those described here. Whether such operations (of 'inference')

provide for the foundations of logical development remains unknown.

BRENDAN O. MCGONIGLE
MARGARET CHALMERS

Department of Psychology,
Edinburgh University,
Edinburgh, Scotland, UK

Received 23 June 1976; accepted 29 April 1977.

- Burt, C. J. *exp. Pedagogy* 5, 68-77 (1919).
- Bryant, P. E. & Trabasso, T. *Nature* 232, 456-458 (1971).
- McGonigle, B. O. J. *comp. Physiol. Psychol.* 64, 110-113 (1967).
- Navarick, D. J. & Fantino, E. *Psychol. Rev.* 81, 426-441 (1974).
- McGonigle, B. O. & Jones, B. T. *Perception* 6, 213-217 (1977).
- Trabasso, T. in *Minnesota Symposium on Child Psychology* 9 (University of Minnesota Press, Minneapolis, 1975).
- de Boysson-Bardies, B. & O'Regan, K. *Nature* 246, 531-534 (1973).

Dissolved ATP in the sea and its utilisation by marine bacteria

THE biologically labile fraction of the dissolved organic matter (DOM) in the oceans seems to be a chemically complex solution of a wide variety of compounds derived mainly from the contents of cellular metabolic pools released into the seawater after the death and cell lysis of marine organisms. This fraction is of particular geochemical interest because of its importance in understanding the cycling of organic matter in the marine environment. Because of methodological problems arising from the extremely low concentrations of individual compounds, compound-specific analyses have been limited to only a few components of the DOM, such as amino acids¹, lipids², sugars³, and vitamins³. Adenosine-5'-triphosphate (ATP) would be a useful tracer for following the production and fate of labile DOM in seawater as it is a universal component of the cellular metabolic pools of all living organisms. We report here that dissolved ATP (DATP) occurs in seawater in significant concentrations (0.1-0.6 µg l⁻¹) and is utilised rapidly by marine bacteria.

Although the particulate ATP content of marine seston (suspended particles) has been used extensively as a sensitive indicator of microbial biomass in oceans and lakes⁴, the presence of DATP in seawater had not been demonstrated conclusively⁵. This has been due, in part, to the absence of a suitably sensitive method, and, in part, to the general assumption that ATP is so rapidly degraded by transphosphorylases and ATPases after cell death as to preclude its presence in the DOM. We considered that the rate of enzymatic breakdown of DATP in the sea might be negligible because extreme dilution of both the enzymes and the substrate occurs after cell disruption.

DATP concentrations in coastal waters of Southern California and British Columbia, Canada were measured using a modification of the method of Hodson *et al.*⁶, which was developed originally for determination of ATP associated with organisms in marine sediments. Seawater samples (50 or 100 ml) were first filtered through 0.2 µm Nuclepore filters to remove ATP associated with organisms and other particles. Filtrates were acidified to 0.6 N with sulphuric acid. A trace amount of γ-³²P-ATP (less than 1 nCi per sample; 700 Ci mmol⁻¹) was added as an internal standard and the sample was passed through a column of activated charcoal. Adsorbed ATP was then eluted with

5 ml ammoniacal ethanol (ethanol—ammonia—water, 2:2:1 v/v), dried *in vacuo* at 40 °C, and dissolved in 1 ml Tris (tris hydroxymethyl aminomethane) buffer (pH 7.7, 20 mM). Percentage recovery of ATP was determined by radioassaying the concentrates without adding fluor, by Cerenkov Radiation, so that the same sample could be used to determine ATP. ATP was measured by the firefly luminescence method⁴.

It was possible that DATP in seawater samples was an experimental artefact, and that it was introduced into filtrates by cell lysis during vacuum filtration. This was found not to be the case by comparing the filterable ATP with dialysable ATP. Cleaned dialysis bags (molecular weight cut-off 12,000–14,000) were filled with double-distilled water and autoclaved. These dialysis bags were suspended from the Scripps Institution of Oceanography (SIO) pier to a depth of 1 m for 4–24 h. The contents were removed with a sterile syringe and hypodermic needle, and assayed for ATP as above. DATP concentration in the dialysis bags was not appreciably different from that in filtrates ($\Delta P=5$ cmHg). Another check was made by filtering aliquots of seawater samples using a range of pressure differentials (2.5–37.5 cmHg) and measuring DATP in the filtrates. No increase in DATP resulted with increasing ΔP .

The concentration of DATP in the relatively oligotrophic surface seawaters off the coast of Southern California varied from an average high value of 218 ng l⁻¹ off the SIO pier to an average low value of 65 ng l⁻¹ at a distance of 4 miles seaward from Point Loma, San Diego, California. In the highly eutrophic waters of Saanich Inlet, British Columbia, the DATP concentration averaged 466 ng l⁻¹. The distribution of DATP with depth was measured at a station 16 miles offshore from San Diego in the San Diego Trough (Fig. 1). The DATP concentration in the euphotic zone averaged 110 ng l⁻¹, decreasing to a uniform value of 40 ng l⁻¹ in the aphotic zone down to a depth of 500 m. At this depth, the ATP content of the particles, including the living organisms, was only 4 ng l⁻¹, which is typical of these waters⁴.

Possible sources of the DATP in surface seawater include phytoplankton mortality due to zooplankton grazing and the attendant release of soluble metabolic pools, and the excretion of ATP by plankton. Possible sources of DATP in the deeper parts of the water column are oceanic mixing processes, and the sinking of faecal pellets, clay minerals, and other particles which may contain associated ATP.

Three possible mechanisms of DATP turnover were examined: spontaneous ATP hydrolysis in seawater, enzymatic hydrolysis by extracellular ATPases, and biological uptake and assimilation. ATP was stable in seawater that had been filtered through 0.2 μ m Nuclepore filters to remove all organisms and then autoclaved (15 min at 121 °C) to denature any ATPases; no loss was detectable after 24 h at 21 °C. The stability was not determined over longer periods of time, but previous work has shown that the half life of ATP in artificial seawater at 21 °C is about 8 yr (ref. 7).

Enzymatic hydrolysis of DATP in seawater was examined by filtering seawater samples through 0.2 μ m Nuclepore filters and following the rate of ATP loss at 21 °C; no detectable loss was observed after a 24 h period.

Rapid turnover of DATP occurs when living organisms are not removed from seawater samples. Turnover rates were determined by measuring the assimilation of 2,8-³H-ATP by the natural microbial populations present in the samples. Controls, which received 1% formalin before addition of ³H-ATP, accumulated (adsorbed) ³H at less than 5% of the experimental samples. Using U-¹⁴C-ATP as tracer, it was determined that more than 98% of the ¹⁴C taken up was assimilated, whereas only an insignificant fraction was respired. Turnover rates varied as expected, but were quite rapid in all samples, with rates

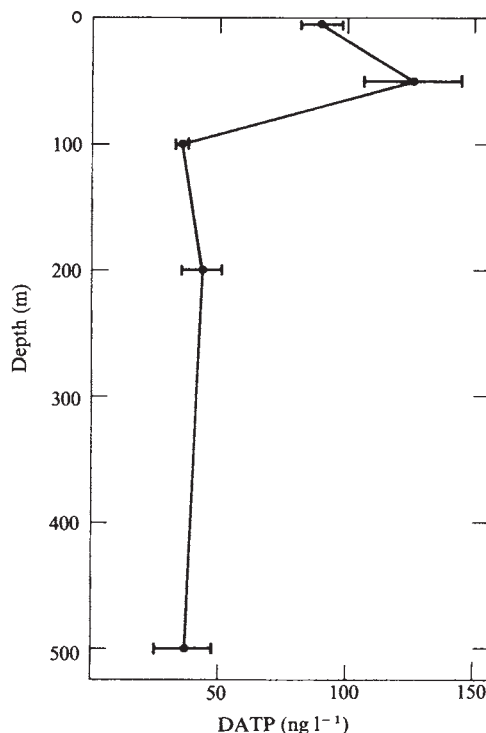


Fig. 1 Dissolved ATP concentration as a function of depth from 32°41.6N; 117°22.7W in San Diego Trough.

as high as 15.2% h⁻¹ in a sample collected in Saanich Inlet, British Columbia; the range was 0.59–15.2% h⁻¹.

To estimate what part of the ATP uptake was due to bacteria, the seawater samples were first incubated with ³H-ATP and then equal aliquots were filtered on 0.2 μ m and 0.6 μ m Nuclepore filters, and the ATP assimilation by total and 0.6 μ m filterable organisms determined. It has been shown previously that the assimilation of radiolabelled organic compounds from seawater by 0.6 μ m filterable particles can be treated as being of bacterial origin⁸. About 80% of the assimilation of DATP was bacterial; the rest is assumed to be due either to algae and micro-zooplankton, or to bacteria attached to larger particles. We have previously shown that bacteria are responsible for 80–90% of the assimilation of dissolved D-glucose, L-serine, and acetate from seawater⁸.

Twelve isolates of marine bacteria were examined for their ability to assimilate 2,8-³H-ATP. Eleven of these isolates were highly efficient in assimilating DATP. Bacterial growth in the sea is characterised by extreme limitation of carbon and energy sources, individual growth substrates being present at nanomolar concentrations. The presence of a significant concentration of DATP which is rapidly turned over suggests that ATP may be an important carbon and energy source for marine bacteria. Its ubiquitous presence in seawater and the fact that its concentration can be determined with great sensitivity (10⁻¹¹ M), may make the measurement of its flux into marine bacteria a useful indicator of bacterial heterotrophic activity in a given body of seawater.

We thank E. Moussalli for technical assistance. This work was supported by ERDA and IDOE/NSF.

F. AZAM
R. E. HODSON

*Institute of Marine Resources,
Scripps Institution of Oceanography,
University of California, San Diego,
La Jolla, California 92093*

Received 14 February; accepted 27 April 1977.

¹ Lee, C. & Bada, J. L. *Earth planet. Sci. Lett.* 26, 61–68 (1975).

- ² Williams, P. M. J. *Fish. Res. Bd Can.* 22, 1107–1122 (1965).
³ Provasoli, L. & Carlucci, A. F. in *Algal Physiology and Biochemistry* (ed. W. D. P. Stewart) 741–789 (Blackwell Scientific, Oxford, 1974).
⁴ Holm-Hansen, O. in *Estuarine Microbial Ecology* (ed. H. Stevenson & R. R. Colwell) 73–89 (University of South Carolina Press, Columbia, South Carolina, 1973).
⁵ Vaccaro, R. F., Hicks, S. E., Jannasch, H. W. & Carey, F. G. *Limnol. Oceanogr.* 13, 356–360 (1968).
⁶ Hodson, R. E., Holm-Hansen, O. & Azam, F. *Mar. Biol.* 34, 143–149 (1976).
⁷ Hulett, H. R. *Nature* 225, 1248–1249 (1970).
⁸ Azam, F. & Hodson, R. E. *Limnol. Oceanogr.* 22, 492–501 (1977).
⁹ Cited in the discussion of: Hodson, R. E., Holm-Hansen, O. & Azam, F. in *ATP Methodology Seminar* (ed. Borun, G. A.) 394–425 (SAI Technology Company, San Diego, California, 1975).

Sex pheromone of the California red scale, *Aonidiella aurantii*

THE California red scale, *Aonidiella aurantii* (Maskell), is the most important citrus pest in Australia, California and the Mediterranean countries. It has been found¹ that red scale females attract males with a sex pheromone, and preliminary studies² have indicated that a branched acetate may be involved. Much interest has been generated in the identification of this pheromone because no other Homopteran sex pheromone has been reported and because the synthetic pheromone has immediate application in replacing the commercial virgin female traps used extensively in monitoring this pest. The sex pheromone reported here consists of two novel components, 3-methyl-6-isopropenyl-9-decen-1-yl acetate (I) and (Z)-3-methyl-6-isopropenyl-3, 9-decadien-1-yl acetate (II). The norsesquiterpene structures represent interesting biosynthetic pathways since they do not merely involve head-to-tail coupling of the isoprenoid units. These compounds also present a synthetic challenge because (I) possesses two asymmetric centers and (II) possesses one asymmetric centre (Fig. 1).

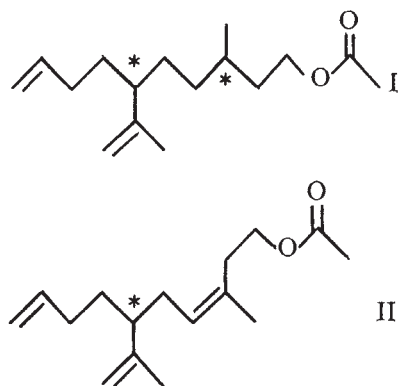


Fig. 1 Components of sex pheromone from California red scale.

Crude pheromone was first obtained from condensates from air that had passed over scale-infested lemons into a dry-ice trap. Steam distillate from the aqueous condensate was extracted with ether-hexane (3:1). An improved system later utilised a Porapak Q trap³ and scale-infested potatoes. A total of approximately 400 million female-day-equivalents (FDE) were collected. An FDE is the amount of pheromone collected from one female each day.

Pentane extracts of the Porapak were concentrated and the residue was chromatographed on a Florisil column (1.5 × 50 cm) eluted with 900 ml of a 0–50% gradient of ethyl ether in Skelly B. The fractions (15 ml) were tested for activity in a greenhouse bioassay⁴, in which test materials on filter paper disks were positioned on a 1.8-m diameter turntable, and red scale males were selectively captured on sticky cards (7.5 × 12.7 cm) placed above each sample. A typical test with Florisil fractions resulted in captures of several hundred males with fractions 14–20 and less than three males with any other fraction.

Active fractions were combined and chromatographed on a 10% AgNO₃-Bio-Sil A (20–44 μm) HPLC column (1 × 100 cm)

eluted with 500 ml each of 10% ether and 20% ether in benzene. Bioassay results showed activity in fractions (15 ml each) 9–14 and 29–38. Each set of active fractions was combined and labelled AI and AII, respectively.

AI and AII were purified further by elution from a Lichrosorb HPLC column (0.2 × 50 cm) with 20 ml 1% ethyl acetate in heptane (0.5 min fractions). All activity was collected during the period 7.5–8.0 min for both samples. Collections from gas-liquid chromatography (GLC) columns showed that the activity was associated with one visible peak in each case, with carbon numbers relative to saturated acetates as follows: 3% OV-101 (AI = 12.25; AII = 12.06); 10% XF-1150 (AI = 13.08; AII = 13.00). The components were purified finally by collection from OV-101 to provide approximately 50 μg of each.

The presence of an acetate moiety was indicated by reaction of AII with LAH to yield an inactive product. Activity was restored at the original GLC retention time on OV-101 by treating the product with acetyl chloride. Mass spectra of AI and AII showed M-60 fragments at 192 and 190 m/e, respectively, possibly corresponding to doubly and triply unsaturated 14-carbon acetates. Hydrogenation produced the same product with an M-60 fragment at 196 m/e from both AI and AII; this was converted to the corresponding hydrocarbon skeleton by saponification, substitution of the hydroxyl group with bromine and reaction with LAH. This hydrocarbon exhibited a molecular ion at 198, a major fragment at 155 (M-43), and typical hydrocarbon fragmentation to the base peak at 57 m/e. Ozonolysis of AI did not yield any GLC visible product, but ozonolysis of AII produced an isolable compound identified as 3-ketobutan-1-yl acetate by GLC and mass spectral comparisons with a standard.

The PMR spectrum of AI (C₆D₆; 100 MHz) was consistent with structure I and has δ values as follows: 5.75 (m), 5.07 (d), 5.02 (d) [H₂C=CH-CH₂-]; 4.82 (s) and 4.76 (s) [R₂C=CH₂]; 4.09 (t) and 1.7 (s) [-CH₂-O-CO-CH₃]; 1.95 (m) [3 allylic H]; 1.49 (s) [CH₃C(R)=]; 1.1–1.6 (m) [9 saturated H]; and 0.77 (d) [-CH-CH₃].

The spectrum of AII (CS₂; 300 MHz) was consistent with structure II and has δ values as follows: 5.71 (m), 4.92 (d), 4.87 (d) [H₂C=CH-CH₂-]; 5.12 (t) [R₂C=CH-CH₂-]; 4.7 (s) and 4.64 (s) [R₂C=CH₂]; 3.95 (t) and 1.92 (s) [-CH₂-CH₃-O-CO-CH₃]; 2.27 (t) [C=C(R)-CH₂-CH₂-]; 1.9–2.1 (m) [5 allylic H]; 1.69 (s) and 1.60 (s) [2 CH₃ C(R)=C]; and 1.39 (m) [2 saturated H].

A 1:1 mixture of E and Z isomers of compound II was prepared from either (S)-(+) -carvone or (R)-(-) -carvone. The former produced the R enantiomer (R:S ratio was 95.5:4.5) and the latter produced the S enantiomer (S:R ratio was 96:4). The geometrical isomers were easily separable on several GLC columns, and were characterised by the chemical shifts (CS₂; 100 MHz) of the 3-methyl proton singlet at 1.69 and 1.60 p.p.m. for the Z and E isomers, respectively. The natural compound AII had GLC retention times identical to the synthetic Z isomer on OV-101 and XF-1150, with no evidence for the E isomer. The proton magnetic resonance spectrum of synthetic II (CS₂; 100 MHz) was similar to that of the natural compound (CS₂; 300 MHz), and mass spectra were identical for AII and II, for their hydrogenated products, and for the hydrocarbon skeletons obtained from each.

Greenhouse flight tests showed that the (R)-Z isomer (25–130 ng on filter paper) is competitive with 25 virgin females, whereas the other isomers are inactive. A typical test (25-ng sample, two replicates for two nights) gave male catches of 531 for the (R)-Z isomer and 15, 17, 18 and 15 for the (R)-E, (S)-Z, (S)-E isomers, and the blank, respectively. Preliminary field tests confirmed the strong attractancy of synthetic II (R-Z) compared with standard virgin female traps (200 females per trap). Field studies will be carried out on all enantiomers of II, as well as with those of I when available.

We thank E. Verwiel, TNO, Delft, The Netherlands for the 300-MHz spectrum, and R. M. Silverstein, Syracuse, New York,

for the 100-MHz spectrum of AI. This research was supported by a USDA cooperative research grant.

W. L. ROELOFS
M. J. GIESELMANN
A. M. CARDÉ
H. TASHIRO

New York State
Agricultural Experiment Station
Geneva, New York 14456

US Department of Agriculture,
Riverside, California 92502

D. S. MORENO

C. A. HENRICK
R. J. ANDERSON

Zoecon Corporation
Palo Alto, California 94304

Received 24 January; accepted 28 April 1977.

- ¹ Tashiro, H. & Chambers, D. L. *Ann. Entom. Soc. Am.* 60, 1166–1170 (1967).
² Warthen, J. D., Jr, Rudrum, M., Moreno, D. S. & Jacobson, M. J. *Insect Physiol.* 16, 2207–2209 (1970).
³ Byrne, K. J., Gore, W. E., Pearce, G. T. & Silverstein, R. M. *J. Chem. Ecol.* 1, 1–7 (1975).
⁴ Tashiro, H., Chambers, D. L., Moreno, D. & Beavers, J. *Ann. Entom. Soc. Am.* 62, 935–940 (1969).

Thermal selection of allozyme polymorphisms in barnacles

Is protein polymorphism adaptive or neutral¹? Attempts to assess the neutrality theory on the basis of gene frequencies and theoretical population genetics models seem, at least to some authors, to be unsuccessful². Possible promising approaches involve the search for direct correlation of isozymes with the environment¹ and with physiological function³. We have tested the effects of temperature on barnacles, because temperature affects protein structure and function^{4,5,6}, as well as size⁷, and because adult barnacles, following larval settlement, are stationary and directly exposed to local temperature variation. Our results suggest strong temperature selection on allozymic and size variations in the acorn barnacle, *Balanus amphitrite*.

Acorn barnacles, genus *Balanus*, are marine crustaceans, with almost worldwide distribution. Our sampling areas were the open Mediterranean seawater canals of the Haifa Electrical Plant Cooling System, where temperature seems to be the only environmental factor distinguishing the cooler inflowing and warmer outflowing canals (about 12 °C and 9 °C mean difference in summers 1975 and 1976, respectively; see Tables). We compared *B. amphitrite* from the cooler and warmer canals for numbers, size, and for allozyme variation at

11 scorable gene loci in 1975 and at 8 differentiating loci in 1976. To compare equal age populations in the cooler and warmer canals, we immersed 625 cm² plastic plates, 100 cm below water surface, in May and removed them in October. A sample of 260 barnacles—six samples, three from the inflowing and three from the outflowing canals—were taken in three repetitive tests, one in 1975 and two in 1976 (from two different pairs of canals, see Tables). Animals that settled on the plates in each outflow have all passed through the relevant inflow. Soft tissues of whole animals were extracted from the calcareous shells, homogenised, and studied using starch gel electrophoresis⁸ for allozymic variation encoded by the following 11 gene loci: phosphoglucose isomerase (*Pgi*); malic enzyme (*Me*); acetaldehyde oxidase (*Ao*); two loci, *Ao-1* and *Ao-3*; malate dehydrogenase (*Mdh-1*); acid phosphatase (*Acph-1*) and esterases (five loci: *Est-1*, *Est-3*, *Est-4*, *Est-7* and *Est-8*).

The number of barnacles per plate (on both sides of the plate) in the inflowing canals was about 2,400 compared with 510 per plate in the outflowing canals. The average size of an individual in a sample from the inflowing canals was 336 mm³ (s.d. 104), compared with 135 mm³ (s.d. 47) in the outflowing canal ($n_1 = n_2 = 30$; $t = 9.65$; $P < 0.001$). Out of 11 isozymes tested in 1975, three (*Ao-1*, *Est-1* and *Est-7*) showed no differences between the cooler and warmer canals, and were therefore discarded. The remaining eight showed distinct repetitive trends in the 1975 and 1976 tests. First, the estimates (Table 1) of heterozygosity (h) for individual loci decreased significantly in four loci (*Me*, *Acph-1*, *Est-3* and *Est-4*), most remarkably in *Me*. Mean heterozygosity for all 8 loci (H) in the three tests decreased significantly from 0.110 in the cooler to 0.047 in the warmer canal ($P < 0.001$). Second, distinct statistically significant repetitive trends (Table 2), in allele frequencies were found for chosen alleles in seven out of the eight loci. The following alleles increased in frequency in the warm canal: *Me*^{M+}, *Ao-3*^F, *Mdh-1*^F, *Acph-1*^M, *Est-3*^F, *Est-4*^F and *Est-8*^F; there was a non-significant increase in *Pg*^S. Significant deviations from Hardy–Weinberg expectations due to heterozygote deficiencies were found for *Me* in both the cooler and warmer canals ($\chi^2_{(1)} = 10.04$, $P < 0.01$; $\chi^2_{(1)} = 79.6$, $P < 0.001$, respectively), and for *Est-3* in the warmer canal only ($\chi^2_{(1)} = 97.8$, $P < 0.001$).

To obtain an approximate measure of the favoured allele we assume fitnesses W_1 , W_2 , W_3 for *AA*, *Aa* and *aa*, respectively, where *a* denotes the favoured allele in the warmer canal, and *A* all the remaining alleles. The relative fitnesses of *aa* compared with *AA* (W_3/W_1) in the warmer compared with the cooler canal for *Me*, *Acph-1*, *Est-3* and *Est-8*, were 3.6, 2.2, 21.3, and 4.4, respectively. Evidently, strong directional selection is

Table 1 Estimates of heterozygosity (h) for each of eight loci in *B. amphitrite* from the inflowing and outflowing canals at the Haifa Electrical Plant Cooling System

Month and year:	October 1975					October 1976					October 1976					Pooled Z
No. of test Canals	In 24.1		Out 36.6		Z ⁴	In 24.6		Out 33.1		Z	In (Roughly as in test 2)		Out		Z	
Temperature (°C, mean) ¹	n ²	h ³	n	h		n	h	n	h		n	h	n	h		
Locus	n ²	h ³	n	h	Z ⁴	n	h	n	h	Z	n	h	n	h	Z	
<i>Pgi</i>	29	0.103	28	0.143	—	71	0.042	68	0.103	—	29	0.034	30	0.167	—	
<i>Me</i>	28	0.464	29	0.172	2.37**	72	0.774	65	0.187	6.86†	29	0.724	30	0.133	4.59†	
<i>Ao-3</i>	29	0.000	30	0.000	—	72	0.000	68	0.029	—	28	0.036	30	0.000	—	
<i>Mdh-2</i>	21	0.048	18	0.056	—	72	0.042	68	0.059	—	29	0.000	30	0.067	—	
<i>Acph-1</i>	29	0.000	29	0.000	0.00	72	0.056	64	0.000	1.92*	29	0.103	26	0.038	0.93	
<i>Est-3</i>	27	0.148	26	0.038	1.37	71	0.085	59	0.000	2.29*	28	0.000	27	0.000	0.00	
<i>Est-4</i>	29	0.172	29	0.034	1.73*	71	0.028	66	0.000	1.37	29	0.103	29	0.034	1.04	
<i>Est-8</i>	29	0.000	29	0.069	—	72	0.000	64	0.016	—	29	0.000	28	0.000	—	
Mean	H ⁵	0.095	*	0.026			0.114	*	0.055			0.111		0.041	**	

¹ Mean temperature calculated from daily minimum–maximum recordings.

² *n*, Number of barnacles tested.

³ *h*, Heterozygosity at each locus.

⁴ *Z*, Statistic comparing proportions with a specified characteristic with two independent samples ($Z = \sqrt{\chi^2}$)¹¹.

Wherever *Z* values do not appear in body of Table, *h* in the outflowing canal is higher than *h* in the inflowing canal. Since the test is one-sided these results are obviously non-significant.

⁵ *H* in the cooler and warmer canals were compared by the Mann–Whitney test, carried out on the observed numbers of heterozygotes over the eight loci in each individual. Thus, it is impossible to perform it directly on the data as they appear in this Table. The *P* values were <0.05 for test 1, <0.01 for test 2, <0.001 for test 3.

Levels of significance: **P* < 0.05; ***P* < 0.01; †*P* < 0.001.

Table 2 Comparison of gene frequencies of chosen alleles at eight loci in *B. amphitrite* from the inflowing and outflowing canals at the Haifa Electrical Power Cooling System

Month and year:		October 1975					October 1976					October 1976					Pooled Z
No. of test		1					2					3					
		In		Out			(same pair as in test 1)					(another pair of canals)					
Canals		24.1		36.6			In		Out			In		Out			
Temperature (°C, mean) ¹							24.6		33.1			(roughly as in test 2)					
Locus	allele	n ²	ρ ³	n	ρ	Z ⁴	n	ρ	n	ρ	Z	n	ρ	n	ρ	Z	
<i>Pgi</i>	(S)	58	0.017	56	0.036	0.63	142	0.007	136	0.029	1.39	58	0.017	60	0.033	0.56	
<i>Me</i>	(M+)	56	0.393	58	0.431	0.41	144	0.281	130	0.515	3.96†	58	0.328	60	0.583	2.78**	
<i>Ao-3</i>	(F)	58	0.000	60	0.167	3.25**	144	0.014	136	0.132	3.83†	56	0.018	60	0.100	1.85	
<i>Mdh-2</i>	(F)	42	0.024	36	0.139	1.90	144	0.007	136	0.029	1.40	58	0.000	60	0.050	1.72	
<i>AcpH-1</i>	(M)	58	0.932	58	1.000	2.02*	144	0.937	128	0.969	1.23	58	0.810	52	0.924	1.74	
<i>Est-3</i>	(F)	54	0.111	52	0.519	4.54†	142	0.056	118	0.390	6.61*	56	0.071	54	0.444	4.49*	
<i>Est-4</i>	(F)	58	0.052	58	0.052	0.00	142	0.000	132	0.045	2.56*	58	0.000	58	0.103	2.51*	
<i>Est-8</i>	(F)	58	0.000	58	0.103	2.51*	144	0.042	128	0.102	1.93	58	0.000	56	0.108	2.56*	
																4.04†	

¹ Mean temperature calculated from daily minimum-maximum recordings throughout the experiment.² n , Number of tested genes (twice the number of zygotes).³ p , Frequency of tested allele.⁴ Z , As defined in Table 1. We used two-sided tests.Levels of significance: * $P < 0.05$; ** $P < 0.01$; † $P < 0.001$.

operating in the warmer canal on the four *aa* homozygotes, apparently because of their high thermal fitness. Furthermore, significant linkage disequilibria¹ were found only in the warmer canal between *Ao-3^F*-*Est-8^F* ($D = 0.024$, $P < 0.001$) and *Est-3^F*-*Est-4^F* ($D = -0.021$, $P < 0.01$). Finally, the mean Nei genetic distance⁹ over the three tests between the cooler and warmer canals was $D = 0.036$, providing a genetic identity index (I) of 0.964.

Similar trends in number, size, heterozygosity and allele frequencies in several loci were also detected in the 1975 test in *B. eburneus*, which coexists with *B. amphitrite*. Only six specimens of *B. eburneus*, however, were found in the outflowing canal in 1975, and none in 1976. Obviously, the temperate and subtropical *B. eburneus* is more sensitive than the subtropical *B. amphitrite*, and is largely excluded from the extreme environment of the warmer canal, as is true for many other species. Similarly, extensive studies of the impact of a power plant on the biology of a subtropical estuarine environment in Florida¹⁰ has shown a drastic decrease of species numbers particularly near upper lethal temperatures¹¹. Note that the upper temperature limits in Florida for many plants and sensitive larval stages of some crustaceans is 31–33 °C.

The correlation observed between thermal and allozymic variations suggests that in barnacles some allozymes are better adapted to higher temperatures by the 'multiple variant' strategy of thermal adaptation, in which different variants function optimally at different temperatures¹². Note that, except for *Mdh*, six out of the seven enzymes showing differences between the cooler and warmer canals were either regulatory or variable substrate enzymes. The former relate to metabolic regulations, the latter may affect metabolic rates³. Since evolutionary changes in enzyme-substrate affinity are directed primarily to maintain the controlling function¹³, regulatory enzymes may be selected, particularly at extreme temperatures, to increase thermal tolerance limits. To investigate this hypothesis the biochemical and physiological properties of the selected allozymes should be tested.

The cooler and warmer canals are two temperature environments into which the zygotes produced at random in the sea settle down randomly in large numbers, with selection favouring different alleles in each canal. There is some heterogeneity in the source population (the Wahlund effect), as shown by the significant heterozygote deficiency in the cool canal. The increase in this deficiency in the warmer compared with the cooler canal presumably arises from directional selection alone. Furthermore, since effective population size, N , is in the thousands, the linkage disequilibria indicate that natural selection may be favouring particular allelic associations rather than operating on each locus separately. The disequilibria suggest close association of the joint regulatory loci which

possibly results in metabolic functional coordination of the allelic forms³. The present patterns of population structure, genic variation and allelic associations exclude models of random drift or neutrality and leave selection on integrated metabolic phenotypes as the only explanatory model of survival in the warmer canal.

The idea that genetic variation is related to environmental variation dates back to Dobzhansky¹⁴ and others, was theorised by Levins¹⁴ and tested by Powell¹⁵ and McDonald and Ayala¹⁶. Levins¹⁴ theory of fitness regards heterozygosity as an adaptation to environment heterogeneity and uncertainty. The less divergence and uncertainty in the environment, the lower the heterozygosity. Our evidence using barnacles supports this hypothesis. The warmer environment is presumably more predictable, being biologically marginal near the upper lethal temperature of barnacles, and selecting for a more homozygous and optimal strategy. By contrast, unpredictability is presumably greater in the cooler environment, which favours higher heterozygosity.

Finally, direct evidence that strong thermal selection is indeed operating on barnacles living in the warmer environment is suggested by the following facts: fourfold decrease in numbers and threefold decrease in body size of *B. amphitrite*; almost total elimination of the temperate *B. eburneus*; and exclusion of most other species that abound in the cool canals. Our barnacle data strongly support the hypothesis that both allozymes and size are positively selected by natural selection as adaptation to warm environments; this has also been reported for other organisms^{17–20}. Positive Darwinian selection seems to be the major determinant at the molecular and organismal levels in the origin and evolution of adaptations.

This research was supported by a grant from the US-Israel Binational Science Foundation to E. N. and R. C. Lewontin, a grant from the Haifa Electrical Plant Corporation to E. N. and T. S.; and a grant from the Maritime Center, University of Haifa. We thank the Electrical Corporation for allowing us to conduct field work at their premises, Dr M. Haber for statistical assistance, Dr A. H. D. Brown for commenting on the manuscript and an anonymous reviewer for his constructive comments.

EVIATAR NEVO
TAMAR SHIMONY
MICHAEL LIBNI

Department of Biology,
University of Haifa,
Haifa, Israel

Received 14 February; accepted 15 April 1977.

¹ Lewontin, R. C. *The Genetic Basis of Evolutionary Change* (Columbia University Press, New York, 1974).

² Ewens, W. J. & Feldman, M. W. In *Population Genetics and Ecology* (eds S. Karlin & E. Nevo) (Academic, New York, 1976).

- ³ Johnson, G. B. *Science* **184**, 28–37 (1974).
- ⁴ Hochachka, P. W. & Somero, G. N. *Strategies of Biochemical Adaptation* (Saunders, Philadelphia, 1974).
- ⁵ Somero, G. N. In *Isozymes II* (ed. C. L. Markert) 221–234 (Academic, New York, 1975).
- ⁶ Moon, T. W. In *Isozymes II* (ed. C. L. Markert) 207–220 (Academic, New York, 1975).
- ⁷ Ray, C. J. *Morphol.* **106**, 85–108 (1960).
- ⁸ Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. & Gentry, G. B. *Univ. Texas Publication* **7103**, 49–90 (1971).
- ⁹ Nei, M. *Am. Nat.* **106**, 283–292 (1972).
- ¹⁰ Thorhaug, A., Segar, D. & Roessler, M. A. *Mar. Pollut. Bull.* **7**, 165–169 (1976).
- ¹¹ Thorhaug, A. In *Thermal Ecology* (eds J. W. Gibbons & Saritz, R. R.) (AEC Symp. series, in the press).
- ¹² Somero, G. N. *Am. Nat.* **103**, 517–530 (1969).
- ¹³ Dobzhansky, Th. *Genetics and the Origin of Species* (Columbia University Press, New York, 1951).
- ¹⁴ Levins, R. *Evolution in changing environments* (Princeton University Press, Princeton, New York, 1968).
- ¹⁵ Powell, J. R. *Science* **174**, 1035–1036 (1971).
- ¹⁶ McDonald, J. F. & Ayala, F. J. *Nature* **250**, 572–574 (1974).
- ¹⁷ Koehn, R. K. *Science* **163**, 943–944 (1969).
- ¹⁸ Koehn, R. K., Perez, J. E., Merritt, R. B. *Am. Nat.* **105**, 51–69 (1971).
- ¹⁹ Mitten, J. B. & Koehn, R. K. *Genetics* **79**, 97–111 (1975).
- ²⁰ Johnson, G. B. *Biochem. Genet.* **14**, 403–426 (1976).
- ²¹ Fleiss, J. L. *Statistical Methods for Rates and Proportions* (Wiley, New York, 1973).

Electrical responses of *Stomatoca atra* induced by an extract of *Aequorea aequorea*

HYDROMEDUSAE swim by rhythmically contracting the circular swimming muscles located in the subumbrella. When species such as *Stomatoca atra*¹, *Sarsia tubulosa*^{2,3}, *Proboscoidactyla flavicirrata*⁴ and *Euphysa japonica*² are disturbed, either by electrical or mechanical stimulation of the exumbrellar surface, the tentacles contract, swimming stops and a tetanic contraction of the radial muscles causes an infolding of the bell margin (Fig. 1b). This protective response is called crumpling⁵ and is initiated by all-or-nothing potentials in the nerve-free, exumbrellar epithelium^{1–4}. *S. atra*, a predacious hydromedusa, is atypical in that it crumples both when disturbed and when in the proximity of large numbers of hydromedusae of several other species. The latter response is initiated by a stimulating substance stored in the mesogloea of the other medusae⁶. The substance is found in crude extracts of *Aequorea aequorea*, *Halistaura cellullaria*, *Pholidium gregarium* and *S. tubulosa*, but not in *S. atra* itself. The crumpling response induced by the extract has been suggested to be a feeding response, trapping prey in the infolded margin⁶. By recording the electrical activity during the crumpling response initiated by extracts of *A. aequorea* I have found that this response is coordinated differently from crumpling initiated by electrical or mechanical stimulation.

Suction electrodes placed on the subumbrellar surface of the animal monitored electrical events associated with swimming or crumpling. A crude extract of the stimulating substance contained in *A. aequorea* was prepared by mincing an intact animal with a scalpel blade and collecting the resulting fluid. To induce crumpling a few drops of the extract were added to a chamber containing a specimen of *S. atra* swimming in 4 ml of seawater at 11 °C, the local

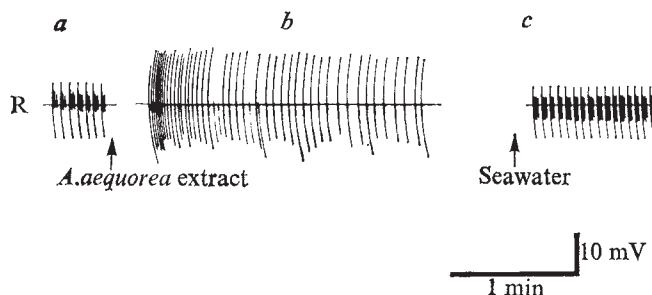


Fig. 2 Electrical activity recorded from the subumbrella of *S. atra*. *a*, Potentials recorded during swimming. *b*, Crumpling potentials induced by the *A. aequorea* extract. *c*, Recovery of swimming potentials after the medium was replaced by fresh seawater.

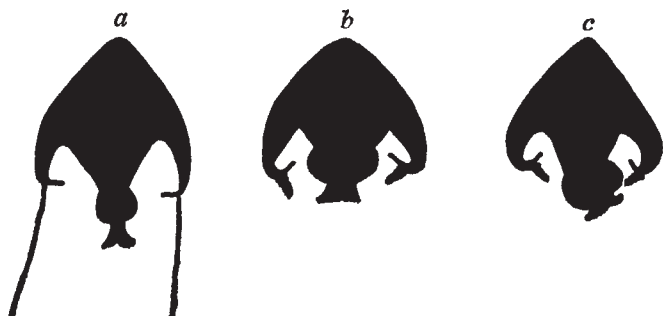
temperature of seawater. Drops of seawater added to the specimen instead of the extract served as a control for all experiments and in no case did seawater induce crumpling. Normally *S. atra* swims in bursts, but in the presence of the extract, the tentacles contracted, swimming and the associated electrical activity ceased and crumpling pulses⁴ were recorded from the subumbrella (Fig. 2). If the solution containing the extract was replaced by fresh seawater the crumpling pulses stopped, the radial muscles relaxed and swimming resumed (Fig. 2).

Since normal crumpling is initiated by propagated potentials in the exumbrellar epithelium, it was important to determine if the extract initiated such electrical events. *A. aequorea* extract did not excite the exumbrellar epithelium of either intact animals or excised pieces of bell containing only exumbrellar tissue. Excised pieces of bell were used as a precaution against recording potentials from tissues deeper than the epithelium directly beneath the active electrode³. The exumbrellar epithelium in both intact animals and excised pieces of tissue did conduct propagated potentials when stimulated electrically and conduction velocity between two electrodes was unaffected by the extract.

The extract not only causes a cessation of swimming and an infolding of the margin but also manubrial flexion⁶, a behaviour not observed in the electrically or mechanically induced crumpling response¹ (Fig. 1b, c). Manubrial flexion is called pointing and is caused by the contraction of one or two of the four radial muscles⁷. Isolated manubria, which included a portion of the peduncle, pointed in the presence of the extract. Suction electrodes placed over a radial muscle, in such a preparation, showed that each flexion was associated with a burst of potentials different from crumpling pulses (Fig. 3). Manubrial flexion stopped when the extract was flushed out with fresh seawater.

The extract-initiated behaviour is similar to normal crumpling but there are differences between the two responses which indicate that they are coordinated differently. I shall refer to the electrically or mechanically induced crumpling as MIC and crumpling initiated by the stimulating substance contained in some hydromedusae as SIC. *S. atra* has six described conducting pathways and MIC has been shown to involve the swimming and tentacle pathways, which are neuronal, and the exumbrellar and lamellar pathways, which are non-neuronal¹. The crumpling pulses initiated by the extract and recorded from the subumbrellar surface were similar in amplitude and duration to the pulses recorded during MIC from the same tissue by Mackie¹. Crumpling pulses recorded from the subumbrella during MIC originate in the endodermal lamella^{1,2}, and by analogy, the crumpling pulses recorded from the subumbrella during SIC are also conducted in the non-neuronal lamellar pathway. Further, the similarity in tentacle shortening in MIC and SIC indicates that the

Fig. 1 Silhouettes of *S. atra* drawn from photographs. *a*, At rest. *b*, During a crumpling response induced by mechanical agitation (MIC). *c*, During a crumpling response induced by the extract of *A. aequorea* (SIC).



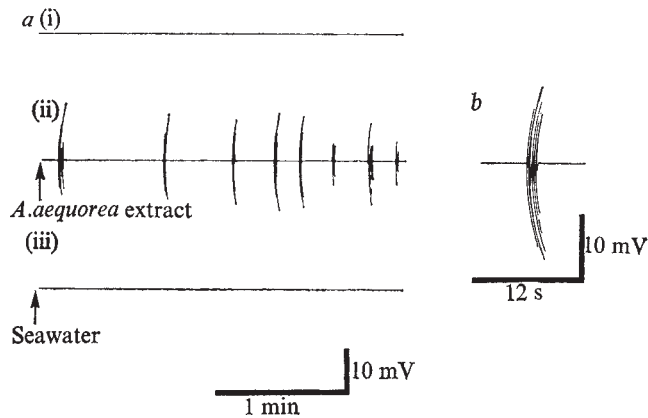


Fig. 3 *a*, Electrical activity from the area of a radial canal in an isolated manubrium (i) before, (ii) during and (iii) after the application of the extracts. *b*, Shows the multiple events associated with the pointing response induced by the extract.

tentacle pathways are used in both behaviours. But MIC and SIIC differ in two aspects. First, SIC does not utilise the exumbrellar pathway as does MIC, since the extract failed to initiate any potentials in that tissue. Presumably the extract excites crumpling through subumbrellar rather than exumbrellar conducting systems. Second, pointing is activated during SIC but not during MIC therefore, SIC involves an additional conducting pathway, called the pointing pathway by Mackie and shown by him to be neuronal¹. There is some evidence that a fourth conducting pathways is involved in SIC. Pointing cannot occur in MIC since all four radial muscles contract tetanically in both the subumbrella and peduncle. In SIC however, the radial muscles in the peduncle do not contract tetanically (note the difference in the peduncular extensions in Fig. 1*b* and *c*) and are free to be used for pointing; therefore, two functionally distinct regions of the radial muscles (subumbrellar and peduncular) are postulated. It is possible that the bridge pathway¹, a conducting system of yet unknown function, may play a role in selectively activating these separate portions of the radial muscles.

I thank Dr A. O. D. Willows, University of Washington Friday Harbor Laboratories, for facilities and Dr R. K. Josephson, University of California, for equipment.

WALTER E. SCHWAB

School of Biological Sciences,
University of California,
Irvine, California 92717

Received 15 November 1976; accepted 22 April 1977.

- ¹ Mackie, G. O. *J. Neurobiol.* 6, 357–378 (1975).
- ² Mackie, G. O., Passano, L. M. & Pavans de Ceccatty, M. C. *r. hebd. Séance Acad. Sci., Paris* 264, 466–469 (1967).
- ³ Mackie, G. O. & Passano, L. M. *J. gen. Physiol.* 52, 600–621 (1968).
- ⁴ Spencer, A. N. *Am. Zool.* 14, 917–926 (1974).
- ⁵ Hyman, L. H. *Biol. Bull.* 79, 282–296 (1940).
- ⁶ Lenhoff, H. M. *Biol. Bull.* 126, 115–120 (1964).
- ⁷ Mackie, G. O. & Singla, C. L. *J. Neurobiol.* 6, 339–356 (1975).

Bivalent-cation-stimulated ATPase activity at preformed exocytosis sites in *Paramecium* coincides with membrane-intercalated particle aggregates

TRICHOCYSTS of *Paramecium* cells, although representing morphologically specialised¹ secretory vesicles, obey the general rules for exocytosis. Expulsion can be triggered by artificial increase of the intracellular Ca^{2+} -concentration^{2,3} ($[\text{Ca}^{2+}]_i$), that is, by Ca^{2+} -mediated stimulus-secretion-coupling^{4,5}; the trichocyst and the cell membrane fuse to form a transient exocytosis canal^{6,7} through which the protein-contents⁸ are discharged. Unlike other secretory

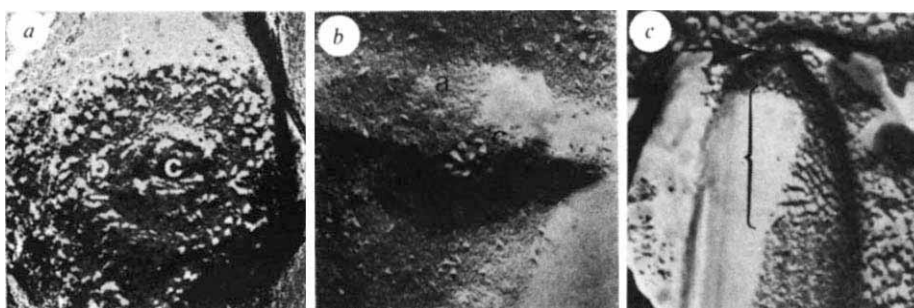
granules, however, trichocysts are, long before discharge, closely attached in a regular pattern to the plasmalemma which, at these attachment sites, contains regular arrays of membrane-intercalated particles^{9,10} (MIP) (Fig. 1*a,b*). Many MIP occur also within the tip region of the trichocyst membrane; Fig. 1*c* also shows some non-etchable 'membrane-connecting material' between trichocyst and cell membrane which probably corresponds to electron-dense materials seen on ultrathin sections⁹. 'Central granule patches' would correspond to 'fusion rosettes' described also for other ciliates¹⁰ and actinopods^{11,12}. The sporadic occurrence of similar MIP aggregates at presumable exo-endocytosis sites of endothelial¹³ and neurohypophysis¹⁴ cells might indicate that *Paramecium* cells are capable of maintaining an otherwise rather ephemeral situation, that is, the stage of membrane-to-membrane attachment preceding membrane fusion. The requirement of energy¹⁵ and Ca^{2+} (refs 4, 5) and the presumable involvement of bivalent-cation-stimulated ATPase activity¹⁶ in the course of exocytosis led us to search for a functional correlate of the specialised structures observed at exocytosis sites.

Paramecium aurelia K 401, was cultivated monoxenically (*Enterobacter agglomerans*) in a standardised Difco medium. For cytochemistry, conducted at 20 °C, cells were washed three times with a ~300-times excess of 10mM Tris-HCl pH 7.0. Washed cells (~2 × 10⁴ per 0.2 ml) were fixed in suspension for 60 s with doubly distilled 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.0 and diluted 1:10 with buffer for 14 min. This method was developed to overcome the marked sensitivity of ATPases to fixation and the discharge of trichocysts by too low fixative concentrations¹⁷. After washing (3 × 5 min) in 10 ml 0.1 M Tris-maleate buffer pH 7.0 cells were incubated for 1 h in the same buffer (eventually at pH 5 or 9) with the following additives: 2.4 mM Pb^{2+} as a capture ion, 5 mM substrates (ATP as Tris salt), 5 mM bivalent and/or 20 mM monovalent cations (chlorides); inhibitors (5 mM) were added also to the last wash before incubation. All chemicals were of the highest purity available and dissolved immediately before incubation. After three washes cells were osmicated (1% OsO_4 in cacodylate buffer pH 7.0, 1 h) (except cells for X-ray microanalysis), dehydrated in acetone and embedded in Durcupan ACM (Fluka). Uncontrasted silver sections were examined in a JEM 100C electron microscope (100 kV). $[\text{K}^+, \text{Na}^+, \text{Ca}^{2+}, \text{Mg}^{2+}]_0$ were determined by atomic absorption photometry (Beckman, model 1248), flame photometry (Instrumentation Lab. model IL 343) and colorimetry in spectral photometers.

Reaction product (RP) was found around the sites where trichocysts are attached to the plasmalemma (Fig. 2) in the following conditions (pH 7): with Pb^{2+} + ATP + Ca^{2+} or Ba^{2+} ; some, but apparently less, RP was also formed with Mg^{2+} . Addition of Mg^{2+} or Na^+ and/or K^+ did not change the reaction obtained with Ca^{2+} . Similarly RP was formed (although independent from the addition of mono- or bivalent cations) with *p*-nitrophenylphosphate, acetylphosphate, Na-AMP and less with Na-β-glycerophosphate as substrates. RP formation was always independent from osmication or section staining. RP was absent at pH 5 and moderate at pH 9 in conditions yielding strong RP at pH 7.

In the following controls no RP was formed (pH 7): omission of Pb^{2+} or chelation by a fourfold excess of 1,2-dihydroxybenzene-disulphonic-(3,5)- Na_2 -salt; omission of appropriate substrates; omission of bivalent cations in presence of Tris-ATP; with Na_2 -ADP ($\pm \text{Ca}^{2+}$), Na_3 -GTP ($\pm \text{Ca}^{2+}$), adenylyl imidodiphosphate (a substrate for the cytochemical assay of adenylate cyclase¹⁸), 3',5'-cyclic AMP or its dibutyl derivative as tentative substrates; after heat exposure during fixation (5 min, 80 °C). Maintaining the original fixative concentration also results in lack of RP. When cells were fixed, soaked in phosphate

Fig. 1 Freeze-cleave replicas obtained with a Balzers BAF 300 (turbomolecular pump; 10^{-6} mbar; 2 min etching at -100°C ; Pt- and C-evaporation by electron beam evaporators). Shadowing direction is from bottom to top. ($\times 72,000$.)



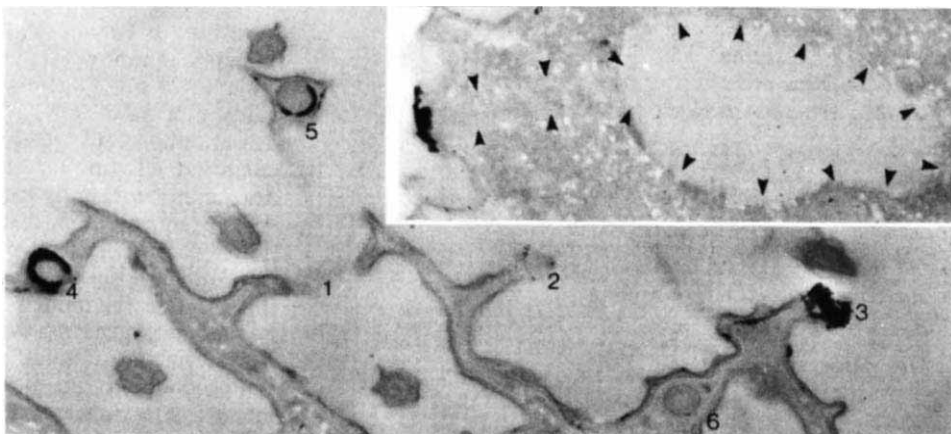
a, Cytoplasmic half of the plasmalemma at a trichocyst attachment site; *a*, 300-nm large double ring⁵ of MIP; *b*, intermediate MIP; *c*, 'central granule patch' of MIP⁶ equivalent to 'fusion rosette'¹⁰. *b*, Outer half of the plasmalemma of a trichocyst attachment site; explanations for *a*, *c* as in Fig. 1*a*. On this fracture plane the 'ring' is represented by pits instead of MIP, whereas about 10 'rosette' MIP (*c*) become better visible on this fracture plane. *c*, Inner half of a trichocyst membrane; note 'annuli'³⁵ of MIP (bracket); corresponding to 'e-type structures' in ref. 6, followed by impressions from the microtubule collar⁶ in the lower portion and non-etchable 'membrane-connecting material' (arrow) between the trichocyst proper and the plasmalemma. For comments on possible structural equivalents of ATPase activity see text.

buffer pH 7 and then in 2.4 mM Pb^{2+} , electron-dense deposits were scattered randomly. Sr^{2+} at pH 9 with subsequent substitution by Pb^{2+} , a method designed for K^{+} , Na^{+} -ATPase¹⁹, gave only spurious RP with various substrate-cation combinations, which resulted in RP formation under 'standard conditions' at pH 7.

At pH 7 media containing Pb^{2+} +Tris-ATP+ Ca^{2+} or Pb^{2+} +*p*-nitrophenylphosphate were used with various inhibitors: ouabain for Mg^{2+} -dependent Na^{+} , K^{+} -stimulated 'transport' ATPase^{20,21}, L-tetramisole for alkaline phosphatase²², and atractyloside for transmembrane nucleotide transport²³. These inhibitors all failed to reduce RP formation. Considerable blockage was obtained with Salycarn or *N*-ethylmaleimide which both inhibit various ATPases^{20,23} by interaction with SH-groups.

The ionic milieu can be characterised as follows: $[\text{K}^{+}]$, $[\text{Na}^{+}]$, $[\text{Ca}^{2+}]$, $[\text{Mg}^{2+}]$ were constantly around 1.8, 0.4, 0.17 and 0.17 mM respectively in the culture medium, 0.05, 0.2, 0.05 and 0.04 before glutaraldehyde fixation and 0.04, 0.04, 0.04 and 0.06 during incubation (without salts added). In living paramecia $[\text{K}^{+}]_i \approx 20$ mM (ref. 24), whereas $[\text{Ca}^{2+}]_i$ probably equilibrates around 0.03–0.05 mM (ref. 25) for our low $[\text{Ca}^{2+}]_o$ values. Assuming that permeability changes due to glutaraldehyde fixation were similar to those in other cells²⁶, we expect from extrapolation a maximum of $[\text{K}^{+}]_i < 10$ mM and $[\text{Ca}^{2+}]_i \approx 0.04$ mM; if the membrane potential generated by high $[\text{K}^{+}]_i$ and $[\text{Ca}^{2+}]_o$ in paramecia²⁴, were to break down in response to the insult of chemical fixation, $[\text{K}^{+}]_i$ and $[\text{Ca}^{2+}]_i$ could be around 0.04 mM at the sites of RP formation (unless when added in excess). In conclusion, with the possible exception of K^{+} , the concentration of the relevant cations can be assumed to be < 0.1 mM, if no salts were added.

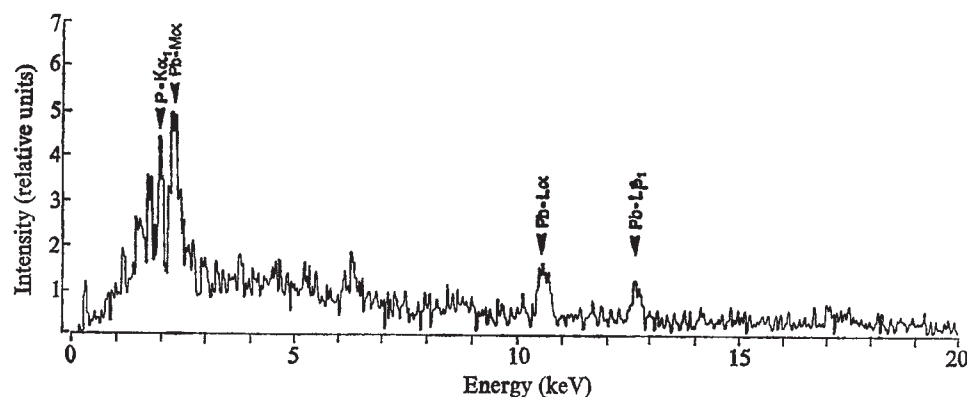
Fig. 2 Cytochemical demonstration of ATPase activity obtained with 5 mM Ca^{2+} + 5 mM Tris-ATP+2.4 mM Pb^{2+} , pH 7, in conditions specified in the text. This 'grazing' section contains several attachment sites of trichocysts (1–6) in a regular pattern (compare with ref. 6). Only where the section contains uppermost tip regions—with structures represented in Fig. 1—reaction product (RP) is deposited. (1–6) Designate a sequence proceeding from the outside to the inside; (1, 2, 6) are unreactive because the sectioning level is still too high (1, 2) or too low (6). On positive sites RP fills the space delineated by its surrounding membranes (see text and ref. 6). *Inset*: Longitudinal section through a trichocyst; outlines are marked by arrows; RP only in tip region. Unstained sections ($\times 31,500$).



To determine whether bivalent cations (5 mM) are truly stimulative we analysed whether Pb^{2+} is the true capture ion in our experiments, since the presence of bivalent cations during glutaraldehyde fixation also results in formation of RP-like deposits on some biomembranes²⁷, including Ca^{2+} -binding sites of paramecia^{3,28,29}. Since X-ray microanalysis revealed the simultaneous presence of P and Ca in such deposits, rapid redistribution of intracellular ATP occurring on fixation²⁶ could lead to cleavage and PO_4^{3-} -capturing by bivalent cations at sites of persisting ATPase activity^{3,27,28}, even if these cations were not stimulative. But, X-ray microanalysis of RP formed with Pb^{2+} +Tris-ATP+bivalent cations revealed the selective enrichment of P and Pb only (Fig. 3). Hence, Ca^{2+} , Ba^{2+} and (to a lesser degree) Mg^{2+} are truly stimulative and Na^{+} , K^{+} non-stimulative ions.

The hydrolysis of various orthophosphate substrates in the absence of additional ions (for K^{+} see above) might be due to a phosphatase which splits phosphorylated intermediates generated by various transport ATPases^{20,21}, including Ca^{2+} -, Mg^{2+} -ATPases^{30,31}. Thus the RP at attachment zones of trichocysts to the plasmalemma probably represents an ATPase stimulated by bivalent cations—in physiological conditions most probably a Ca^{2+} -(transport?)-ATPase.

Various transport ATPases, including Ca^{2+} -ATPase³² are membrane-integrated oligomeric proteins which would show up in freeze-cleaved membranes as MIP of the usual size range³³. Absence of RP from potential attachment sites (unpublished observations) which contain only 'rings' of MIP if not occupied by trichocysts^{3,34} points mainly towards 'central granule patches' (fusion rosettes) and possibly 'membrane-connecting material' and/or 'annuli' as



candidates for the structural equivalent(s) of bivalent-cations-stimulated ATPase. Since RP might diffuse within the small cleft between apposed membranes (plasmalemma, trichocyst and alveolar membrane, see ref. 6), further investigations have to envisage a discrimination among these possible candidates.

Several possibilities have to be considered to explain how Ca^{2+} and ATP could intervene in the final steps of exocytosis (see also ref. 16). The local restriction of RP indicates a mechanism involving local $[\text{Ca}^{2+}]$ -regulation at exocytosis sites. This could entail (1) regulation of membrane repulsion or attraction depending on charge screening, or (2) conformational changes of the 'rosette' MIP or of 'membrane connecting material' reminding the postulated mechanocomplex¹⁸ and the actomyosin-like fibrillar materials occurring at exocytosis sites¹⁵; furthermore (3) Ca^{2+} -dependent regulation of lipid phase transitions³⁵ and intramembraneous segregation phenomena¹⁰ could be involved in the local MIP clustering described.

Trichocyst discharge is a very effective, specialised form of exocytosis, but the structural-functional correlations involved could clarify the final steps of exocytosis in other systems.

We thank Mrs Alice T. Orque for technical assistance, Professor H. Bengner and Dr F. Tiefenbrunner for providing laboratory facilities, and the 'Österreichische Forschungsfonds' for financial support.

Department of Pharmacology,
University of Innsbruck,
Peter-Mayr-Strasse 1

Department of Dermatology,
University of Innsbruck,
Anich-Strasse 4

Department of Hygiene and Microbiology,
University of Innsbruck,
Fritz-Pregl-Strasse 3
A-6020 Innsbruck, Austria

Received 20 January; accepted 21 April 1977.

- 1 Bannister, L. H. *J. Cell Sci.* **11**, 899-929 (1972).
- 2 Plattner, H. *Nature* **252**, 722-724 (1974).
- 3 Plattner, H. & Fuchs, S. *Histochemistry*, **45**, 23-47 (1975).
- 4 Douglas, W. W. *Br. J. Pharmac.* **34**, 451-474 (1968).
- 5 Rubin, R. P. *Calcium and the Secretory Process* (Plenum, New York and London, 1974).
- 6 Plattner, H., Miller, F. & Bachmann, L. *J. Cell Sci.* **13**, 687-719 (1973).
- 7 Plattner, H. *Expl Cell Res.* **103**, 431-435 (1976).
- 8 Steers, E., Beisson, J. & Marchesi, V. T. *Exp Cell Res.* **57**, 392-396 (1969).
- 9 Bachmann, L., Schmitt, W. W. & Plattner, H. *Proc. 5th Eur. Congr. Electron Microsc.* (ed. Cosslett, V. E.) 244-245 (Institute of Physics, London and Bristol, 1972).
- 10 Satir, B., Schooley, C. & Satir, P. *Nature* **235**, 53-54 (1972); *J. Cell Biol.* **56**, 153-176 (1973).
- 11 Bardele, C. F. Z. *Naturforsch.* **31C**, 190-194 (1976).
- 12 Davidson, L. A. *Cell Tissue Res.* **170**, 353-365 (1976).
- 13 Simionescu, M., Simionescu, N. & Palade, G. E. *J. Cell Biol.* **60**, 128-152 (1974).
- 14 Dreifuss, J. J., Akert, K., Sandri, C. & Moor, H. *Cell Tissue Res.* **165**, 317-325 (1976).
- 15 Palade, G. E. *Science* **189**, 347-358 (1975).
- 16 Poste, G. & Allison, A. C. *Biochim. biophys. Acta* **300**, 421-465 (1973).

Fig. 3 Energy spectrogram obtained from reaction product, specified in Fig. 2, by X-ray microanalysis using an analytical electron microscope JEM 100C (60 kV) in the scanning transmission mode and combined with an eucentric goniometer stage (40°), a Kevex detector and a Link spectrometer unit, system 290. Experimental conditions were as before^{3,30} but C-coated nylon-grids (Touzart-Matignon) were used to mount ~1000-Å thick, non-osmicated, unstained sections. Note the occurrence of Pb-lines Ma (2.342 keV), La (10.550), $\text{L}\beta_1$ (12.612) and of $\text{P-K}\alpha_1$ (2.013), while $\text{Ca-K}\alpha$ (3.690) is absent. $\text{Si-K}\alpha$ (1.739 keV) is an artefact.

- 17 Plattner, H., Wolfram, D., Bachmann, L. & Wachter, E. *Histochemistry* **45**, 1-21 (1975).
- 18 Howell, S. L. & Whitfield, M. J. *Histochem. Cytochem.* **20**, 873-879 (1972).
- 19 Ernst, S. A. *J. Histochem. Cytochem.* **20**, 13-22; 23-35 (1972).
- 20 Dahl, J. L. & Hokin, L. E. A. *Rev. Biochem.* **43**, 327-356 (1974).
- 21 Huang, W. & Askari, A. *Archs Biochem. Biophys.* **175**, 185-189 (1976).
- 22 Borgers, M. J. *Histochem. Cytochem.* **21**, 812-824 (1973).
- 23 Vignais, P. V. *Biochim. biophys. Acta* **456**, 1-38 (1976).
- 24 Naitoh, Y. & Eckert, R. in *Cilia and Flagella* (ed. Sleight, M. A.) 305-352 (Academic, London and New York, 1974).
- 25 Browning, J. L. & Nelson, D. L. *Biochim. biophys. Acta* **448**, 338-351 (1976).
- 26 Penttilä, A., Kalimo, H. & Trump, B. F. *J. Cell Biol.* **63**, 197-214 (1974).
- 27 Oschman, J. L., Hall, T. A., Peters, P. D. & Wall, B. J. *J. Cell Biol.* **61**, 156-165 (1974).
- 28 Plattner, H. *J. Cell Sci.* **18**, 257-269 (1975); in *Proc. 6th Eur. Congr. Electron Microsc.* (ed. Ben-Shaul, Y.) 2, 221-222 (TAL International, Tel Aviv, 1976).
- 29 Fisher, G., Kaneshiro, E. S. & Peters, P. D. *J. Cell Biol.* **69**, 429-442 (1976).
- 30 Pucell, A. & Martonosi, A. J. *biol. Chem.* **246**, 3389-3397 (1971).
- 31 Schatzmann, H. J. in *Calcium Transport in Contraction and Secretion* (eds Carafoli, E., Clementi, F., Drabikowski, W. & Margreth, A.) 45-49 (North Holland, Amsterdam, 1975).
- 32 Malan, N. T., Sabbadini, R., Scales, D. & Inesi, G. *FEBS Lett.* **60**, 122-125 (1975).
- 33 Zingsheim, H. P. & Plattner, H. in *Methods in Membrane Biology* 7 (ed. Korn, E. D.) 1-146 (Plenum, New York, 1976).
- 34 Beisson, J., Lefort-Tran, M., Pouphe, M., Rossignol, M. & Satir, B. *J. Cell Biol.* **69**, 126-143 (1976).
- 35 Papahadjopoulos, D., Vail, W. J., Pangborn, W. A. & Poste, G. *Biochim. biophys. Acta* **448**, 265-283 (1976).

Transition probability and the origin of variation in the cell cycle

It is well established that the cell cycle times of cells cultured *in vitro* are highly variable although the origin of this variability has remained unclear^{1,2}. A number of theories have been advanced to explain the variation in cell cycle times although none of them give entirely satisfactory fit to the experimental data². Any proposal as to the origin of this variability must take account of the observation that most of the variability occurs in the G_1 phase of the cell cycle and that it is the length and variability of this phase which is the major determinant of the length and variability of cell cycle times within a cell population¹. A new model of the cell cycle has been proposed independently by two groups that suggests that variability in the G_1 phase of the cell cycle arises as a result of a probabilistic transition occurring within G_1 (refs 3, 4). Using time-lapse cine-photography I have examined the possible role of a number of other factors which might determine this variability and have come to the conclusion that the observed variation in cell cycle times *in vitro* is qualitatively and quantitatively in accord with variation predicted by the transition probability model of the cell cycle⁴.

The transition probability model divides the cell cycle into two parts—the B phase (containing S, G_2 , M and part of G_1) where the cells are proceeding towards division, and the A state (contained in G_1) where the cells are awaiting a random triggering event before proceeding. A cell may remain in the A state for any length of time, but in steady-state conditions the probability (the transition probability) of a cell leaving the A state and entering the B phase in any unit of time is constant. In a population of cells the shortest intermitotic time is shown by a cell that leaves the A state immediately it enters. Intermitotic times greater

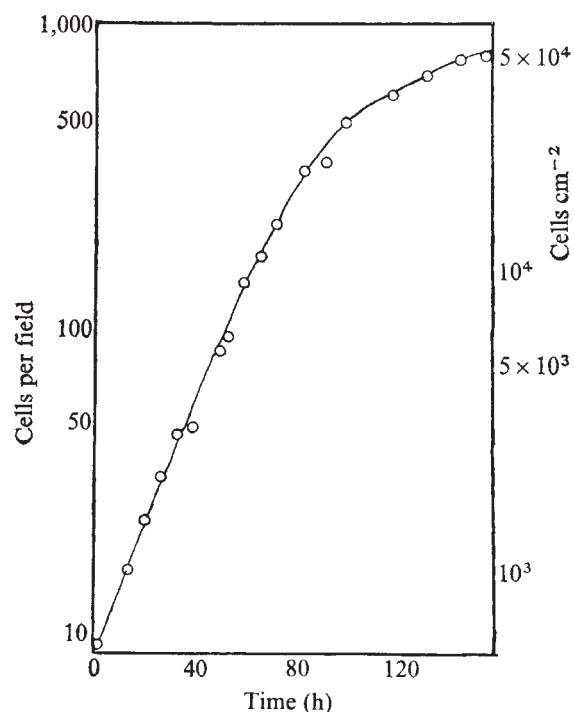


Fig. 1 Growth curve of Swiss 3T3 cells. The cells were plated in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. The cultures were filmed using a 4-min interval and cell counts made from projections of individual frames of the film. The saturation density of these cells is about 5×10^4 cells cm^{-2} .

than this minimum (equal to the duration of B phase, T_B) should be exponentially distributed in a population of cells with identical T_B values and identical transition probability. A graph of the logarithm of the percentage of undivided cells (α_t) against time (the α curve) would show two

components, since the value of α_t will be 100% for times up to T_B and then decay exponentially. In practice the α curves often exhibit some initial curvature that has been ascribed to variation of T_B within a cell population⁴. But, the values of T_B in sibling cells are apparently very similar so that the distribution of differences of intermitotic times of sister cells (the β plot) is also exponential and parallel to the exponential tail of the α curve^{5,6}.

Heterogeneity of cell cycle times approximating to an exponential frequency distribution may be generated in ways other than assuming a random transition. Since most of the observations to date have been on uncloned cell populations, the observed variability could arise from pooling data from cells with differing constitution. To investigate this possibility the behaviour of single clones of the established Swiss 3T3 mouse fibroblast cell line was observed. Cells were plated at low density and filmed until the culture reached confluence (Fig. 1), and α and β curves were constructed generation by generation for eight separate clones for up to six generations (Fig. 2). Subsequent generations were not monitored because cultures were no longer in a steady state over the period of a generation and the cells were increasingly hard to follow so no further data was collected. The α and β curves reveal two types of clonal behaviour. Most of the clones (six of eight) behaved like the clone (clone 5) illustrated in Fig. 2a. The first four generations of this clone were pooled to provide enough data for α and β curves; it made little difference if the early generations were pooled within or between clones (not shown). After some initial curvature the α curves became exponential and closely parallel to the β curve providing evidence that the variation in cell cycle times is not due to clonal heterogeneity. For this clone the minimum value of T_B was 11.6 h in the first four generations, 13 h by the fifth and 14.3 h by the sixth. Similar increases in the value of T_B with successive generations were noted for all the clones examined although T_B varied

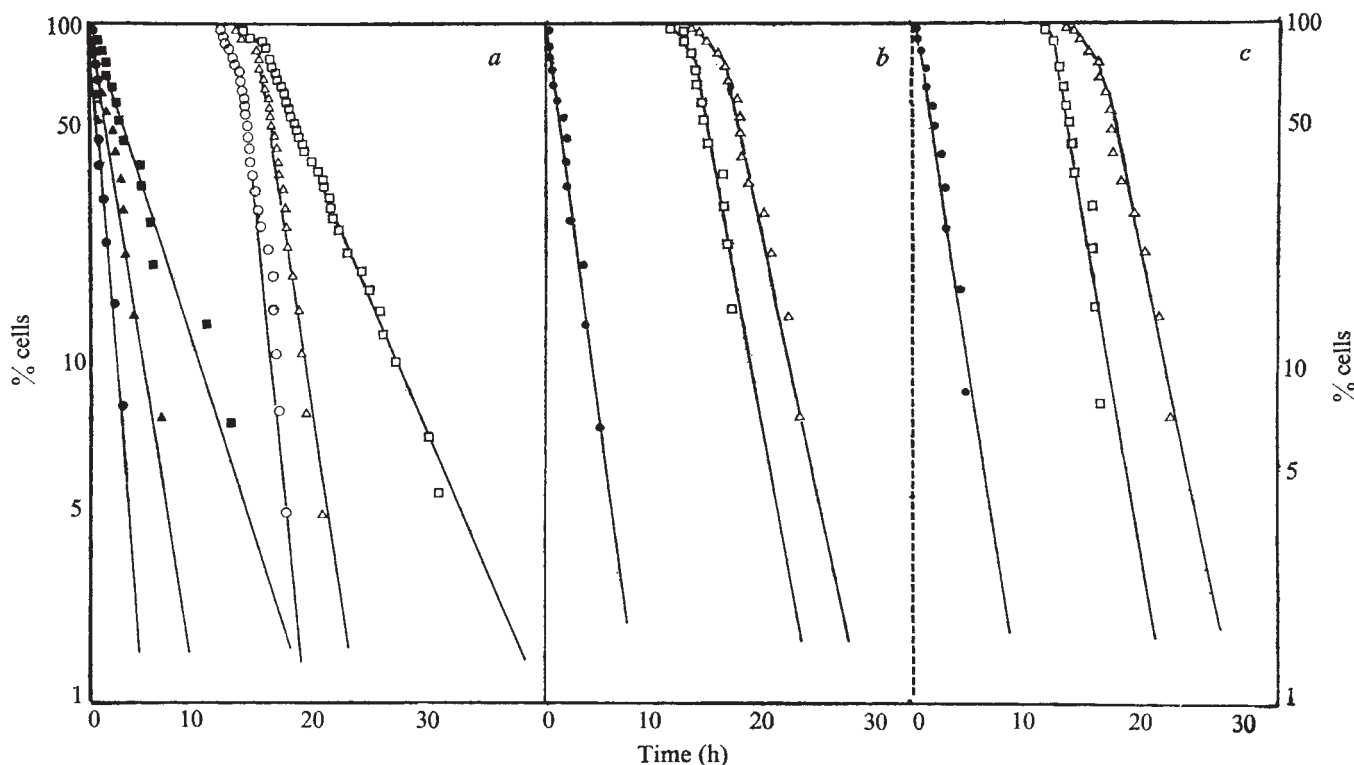


Fig. 2 Semilogarithmic plot (open symbols) of the % of cells (α_t) undivided against age (t) for a clone in the experiment shown in Fig. 1. Also shown (closed symbols) are plots of the % of sibling pairs (β_t) with a difference of intermitotic times $\geq t$. *a*, $\circ$, $\bullet$, Pooled first four generations; $\triangle$, $\blacktriangle$, fifth generation; $\square$, $\blacksquare$, sixth generation (clone 5). *b*, α plots, β plots of the pooled first four generations of another clone (clone 3) from the experiment shown in Fig. 1. $\square$, $\triangle$, Descendents of the two progeny of the first division. The β curves of these two subclones were identical (not shown) and have been pooled for clarity. *c*, As (*b*) but for the fifth generation. The β curve is pooled from both subclones.

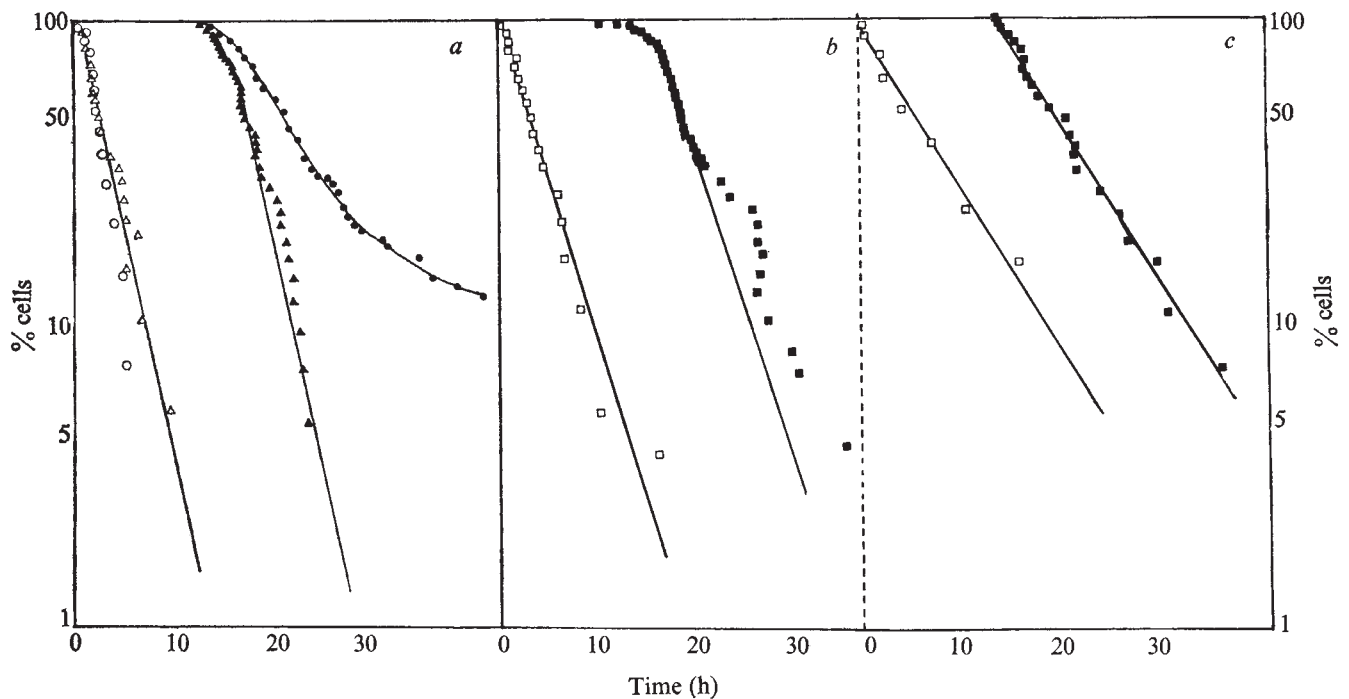


Fig. 3 α and β plots of primary mouse fibroblast (MEF) and primary chicken fibroblasts (CEF). Mouse embryo cells were prepared from whole embryos of Tylers original mice. Cells were plated at 2×10^5 per 5-cm dish in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Chicken fibroblasts were prepared from 10-d embryos of C/O brown leghorn chicks. Cells were plated at 4×10^5 per 5-cm dish in Dulbecco's modified Eagle's medium supplemented with 10% Tryptose phosphate broth, 1% foetal calf serum and 1% heat-inactivated chicken serum. Both cultures were filmed using a 4-min interval. *a*, α and β curves for MEF. Intermitotic times were collected from the progeny of several different cells; $\bullet$, pooled data excluding the three clones shown separately; $\circ$, corresponding β curve. Δ , α Curve from pooled data of three clones in the MEF population; $\triangle$, corresponding β curve for pooled data of three clones. *b*, *c*, α Curves (closed symbols) and β curves (open symbols) for the progeny of two separate CEF cells. In (*b*) the deviation of the points from the drawn α curves is probably due to extensive T_B heterogeneity within a subfraction of the population. Nevertheless, the intermitotic times of 60% of the cells lie on the drawn α curve but the β curve derived from all the cells of the clone is linear.

between the clones and between the generations. Nonetheless, a major cause of the lengthened cell cycle in later generations is the decreased value of the transition probability (estimated from the slope of the β plots).

The other type of clonal behaviour (two out of eight clones) is illustrated in Fig. 2*b* and *c*. In these cases the descendants of the daughters of the first division behave as separate clones differing mainly in the length of T_B . As a consequence the α curves are parallel to each other and the β curves superimposable, this behaviour persists until at least the fifth generation. After this the lifespans of the sixth generation subclones no longer overlapped and the transition probability in the two subclones was different so the T_B difference could not be calculated (not shown). The results from clones of this type show that T_B may (at least in part) be heritable and persists over several generations. This generation of T_B heterogeneity within a clone may be the source of T_B variation within a cell population. Results from the Swiss 3T3 cell line do not support the idea that the apparent exponential component of the variability in cell cycle times within a population arises from clonal heterogeneity as this variability arises even within clones.

It might be argued that karyotypic instability in established cell lines accounts for the variation in cell cycle times even within a cell clone. This possibility has been explored by examining the behaviour of primary cultures of mouse and chicken fibroblasts which are presumably isogenic and diploid. The behaviour of mouse embryo fibroblasts (MEF) is depicted in Fig. 3*a*. The α curve for the pooled data from many different cells (excluding three clones, see below) is sigmoid indicating that a significant fraction of the population did not divide. Nevertheless, a β plot from the same cells is exponential as non-dividing

cells had non-dividing sisters thus giving no value for the difference in intermitotic times. The descendants of some cells could be followed for a number of generations, data from three clones were pooled and α and β plots shown in Fig. 3*a*. The β curve is exponential and parallel to the exponential tail of the α curve. Furthermore, the β curve of the clones is superimposable on the β curve of the pooled data from all the cells which excluded these clones, indicating that the clones are not an unrepresentative fraction of the viable cells. Similar experiments with primary chick fibroblasts (CEF) are illustrated in Fig. 3*b* and *c*. There was considerable clonal heterogeneity in the CEF population so the data could not be pooled. Results from two clones (Fig. 3*b* and *c*) show differences in T_B as well as transition probability, in both cases exponential β curves were obtained.

The α curves shown in this paper rarely extend below the 5% level of undivided cells. This is due to the limited number of cells that can be analysed in a single generation of a single clone. Pooled data from many generations of BALB/c3T3 cells transformed with SV40 virus extended these α plots down to the 0.3% undivided level and the β curve down to the 1% level. The β curve was exponential and parallel to the exponential tail of the α curve⁶.

It could be argued that the exponential kinetics observed is due to escape from some block unwittingly imposed by the conditions of *in vitro* culture. This seems unlikely as free-living organisms such as *Euglena* also have exponentially distributed cell cycle times⁷ as do *Escherichia coli* with cycle times as short as 30 min which are highly unlikely to be blocked (L. Martin, personal communication). Furthermore, the shape of frequency of labelled mitosis curves obtained *in vivo* may be accurately predicted from transition probability theory^{3,4,8-10}, which suggests that cells

in vivo may undergo a similar type of random transition to that observed *in vitro*.

The results in this paper demonstrate that the variability in cell cycle times *in vitro* is not entirely due to pooling data from many cells with different pedigrees nor does it arise from an artefact of using cell lines. These results, together with the fact that the β curves are exponential (which is necessary and a sufficient condition for the existence of a random transition in the cell cycle (R. S. and J. A. Smith, unpublished)), support the idea that the variation in cell cycle times *in vitro* is due in part to a random transition in the cell cycle and that transition probability is a key function that regulates cell proliferation.

I thank Peter Riddle and Diane Brown for filming the cultures, Jenny Beamand for preparing the CEFs and the staff of the cell production unit for preparing the MEFs.

ROBERT SHIELDS

Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2, UK

Received 3 February; accepted 12 May 1977.

- ¹ Sisken, J. E. & Kinoshita, R. *J. biophys. biochem. Cytol.* 9, 509–518 (1961).
- ² Dawson, K. B., Madoc-Jones, H. & Field, E. O. *Expt Cell Res.* 38, 75–84 (1965).
- ³ Burns, F. J. & Tannock, I. F. *Cell Tissue Kinet.* 3, 321–324 (1970).
- ⁴ Smith, J. A. & Martin, L. *Prod. natn. Acad. Sci. U.S.A.* 70, 1263–1267 (1973).
- ⁵ Minor, P. D. & Smith, J. A. *Nature* 248, 241 (1974).
- ⁶ Shields, R. & Smith, J. A. *J. cell. Physiol.* (in the press).
- ⁷ Smith, J. A. & Martin, L. in *Cell Cycle Controls* (eds Padilla, G. M., Cameron, I. L. & Zimmerman, A.), 43–60 (Academic, New York and London, 1974).
- ⁸ Lee, A. E., Rogers, L. A. & Trinder, G. J. *Endocr.* 61, 117–121 (1974).
- ⁹ De Maertelaer, V. & Galand, P. *Cell Tissue Kinet.* 8, 11–22 (1975).
- ¹⁰ De Maertelaer, V. & Galand, P. *Cell Tissue Kinet.* 10, 35–42 (1977).

Hybrid cell lines with T-cell characteristics

THE hybridisation of established cell lines with differentiated cells provides a useful strategy for the production of cell lines having differentiated properties and unlimited growth potential. Clones of these hybrids could be particularly useful in the dissection of cellular interactions of the immune system, a network involving numerous specific cell types which display characteristic ensembles of surface and internal markers. We describe, here, the production and partial characterisation of hybrid cell lines with surface antigens characteristic of T lymphocytes.

Köhler and Milstein^{1,2} have shown that the fusion of mouse myeloma cells cultivated *in vitro*, with spleen cells from immunised mice, gives rise to B-cell hybrid cell lines which secrete monoclonal immunoglobulins with antibody activity to the immunising antigen. Although 30–40% of the spleen cells used for fusion were T cells, none of the hybrids expressed the Thy-1 surface antigen, a characteristic marker for mouse T cells. Without expression of this (or some other) T-cell marker, it is difficult to determine in retrospect whether the non-producing hybrids were T cells-myeloma fusion products in which Thy-1 is repressed.³

In contrast with results from the myeloma fusion studies, we found that fusion of spleen cells with a mouse T-lymphoma cell line (BW 5147) gives rise to hybrid cell lines which express T-cell and not B-cell markers. Hybrid populations were obtained by fusing spleen cells from (BALB/c×SJL)_F₁ mice with HAT-sensitive BW 5147-AzG^R, Ou^R cells (kindly provided by Dr Robert Hyman). The BW 5147 cells were HGPRTase negative and resistant to 1×10^{-3} M ouabain. Spleen donors were immunised by intraperitoneal injection of 100 μ g of DNP-KLH on alum with 2×10^7 *Bordetella pertussis*, and boosted 3 months later by intravenous injection of 20 μ g of DNP-KLH. Cells were harvested from the spleens 4 days later. Hybrids were also obtained by fusing BW 5147 with spleen cells from non-immunised CBA and AKR donors which had been stimu-

lated for 4 or 5 d in mixed lymphocyte cultures (MLC) by irradiated allogeneic spleen cells.

For the fusion, a mixture of 10^8 spleen or MLC-derived cells and 10^7 BW 5147 cells in Dulbecco's Modified Eagles medium plus 10% foetal calf serum (DME-FCS) was pelleted at 400g. The medium was removed and fusion initiated by the gradual addition of 2 ml of 50% (w/w) polyethylene glycol (PEG) 1,000 or 1,500 in serum-free DME. After 2 min at 37 °C, the suspension was then gradually diluted to 50 ml (over the course of 10 min) with serum-free DME and then pelleted by centrifugation⁴.

The pelleted cells were next resuspended and cultured in 0.2- or 2-ml volumes at $1-2 \times 10^6$ cells ml⁻¹ in selective medium (DME-FCS, 100 μ M hypoxanthine, 10 μ M aminopterin, 30 μ M thymidine and 3.3×10^{-4} ouabain). Preliminary experiments have shown that neither spleen cells nor BW 5147 cells survive for longer than a few days in this medium; therefore, after 14 d, selective medium was removed and replaced with DME-FCS. All populations surviving after 2 weeks were presumed to be hybrids since survival requires that the gene for HGPRTase from the spleen cell parent and the (genetic) information for continuous growth from the lymphoma parent both be present in each cell.

In later experiments, cell numbers per well were adjusted to allow one or a few hybrid clones to be seeded. Hybrid populations were transferred when adequate growth was observed. Clonal populations were obtained either by limiting dilution in 0.25-ml wells or by soft agar cloning. Only the (BALB/c×SJL)_F₁ hybrid populations were cloned at the time of this report.

The putative hybrids were analysed for DNA content per cell using flow micro fluorometry in a fluorescence activated cell sorter (FACS-II, Becton-Dickinson Electronics Laboratory, Mountain View, California) after fluorescent straining of the DNA with propidium iodide in hypotonic (0.1%) sodium citrate⁵. Approximately 40 hybrid populations were tested within the first 2 weeks after hybridisation. All had G₁ DNA contents which were about the sum of the DNA contents of spleen cell (diploid mouse) and the near tetraploid lymphoma used for hybridisation.

The expression of both parental Thy-1 alleles on 29/39 hybrid cell populations (and clones) obtained from hybridisation of Thy-1 differing cells demonstrates directly that the hybrids arose from fusion of normal spleen cells and the BW 5147 lymphoma (see Table 1). Specificity of typing was confirmed by quantitatively absorbing the anti Thy-1.2 with hybrid cells and then testing the absorbed antiserum on normal thymocytes to demonstrate removal of anti Thy-1.2 activity. The demonstration that hybrids of BW 5147 with a Thy-1.1 carrying strain, AKR, express only Thy-1.1 rules out expression of a cryptic Thy-1.2 allele by the hybrids (Table 1).

Identification of the splenic lymphocyte subpopulation which fused with BW 5147 to give rise to hybrids expressing both parental Thy-1 alleles is difficult in retrospect; however the increased frequency of such hybrids obtained by fusion of MLC cells, which are nearly all T cells, suggests that the Thy-1.1, 1.2 hybrids are probably derived from fusions of normal T cells with the lymphoma (Table 1). Whether the fusions which gave rise to hybrids expressing only the BW 5147 Thy-1.1 allele were also T cell-lymphoma fusions may become clear as the hybrids are tested for more T-cell markers. Interestingly, the spleen cell hybridisation gave rise to two clones which express neither parental Thy-1 allele, that is in which the BW 5147 Thy-1.1 is lost or repressed.

Preliminary tests for Ly 1,2,3, and Ia markers on the hybrids are consistent with hybrids being derived from fusions of normal T cells and lymphoma cells. The BW 5147 lymphoma comes from a strain which carries

Table 1 Expression of Thy-1 antigens on hybrid cells

Experiment	Hybridisation	Parental populations	Number of hybrid populations expressing Thy-1 alleles*			
			1.1+1.2	1.1	1.2	—
921	AKR T Lymphoma BW 5147 (Thy-1.1)	× (BALB/c × SJL)F ₁ Spleen (Thy-1.2)	18	7	0	2
C.2	AKR T Lymphoma BW 5147 (Thy-1.1)	× CBA Spleen MLC† (Thy-1.2)	11	1	0	0
A.2	AKR T Lymphoma BW 5147 (Thy-1.1)	× AKR Spleen MLC (Thy-1.1)	0	13	0	0
Kohler and Milstein ⁴	BALB/c Myeloma MOPC 21 (X63) (Thy-1 negative)	× BALB/c Spleen (Thy-1.2)	0	0	0	> 10

*Populations from experiment 921 are individual clones, populations from experiments C.2 and A.2 are isolates from different fusion hybrids and may contain one or more clones. Thy-1 phenotype was determined by direct cytotoxicity using congenic anti-Thy 1 antisera (experiment 921) and non-congenic antisera (experiments C.2 and A.2). Thy-1 phenotype of several clones in experiment 921 was confirmed by quantitative absorption studies.

†Cells from a 5-d mixed lymphocyte culture (MLC) of spleen cells stimulated with irradiated allogenic cells. Nearly all surviving cells in this culture are T cells from the responder spleen.

Ly-1.2 and Ly-2.1; however it expresses neither of these characteristic T-cell markers. T cells from (BALB/c × SJL)F₁ mice carry Ly-1.2 and Ly-2.2 determinants. Some of the clones derived from the (BALB/c × SJL)F₁ hybridisation selectively express Ly-1.2 determinants, some selectively express Ly-2.2 determinants while the majority expresses neither. Similarly, Ia determinants selectively expressed on a very small subpopulation of lymphocytes (most likely T) in Ia^b haplotype mice and not on BW 5147 (Ia^k) are expressed in some of the hybrid clones derived from the (BALB/c × SJL)F₁ Ia^d/Ia^s haplotype spleen cells. Among normal T lymphocytes, selective expression of Ly and Ia determinants defines supopulations responsible for the various T-lymphocyte functions^{6,7}.

From the above, it is clear that the hybridisation of spleen cells with an appropriate established cell line (such as the BW 5147 T lymphoma) can yield continuous cell populations expressing characteristic T-cell surface markers. The preliminary evidence for expression of functional T-cell subset markers by these hybrid populations encourages us to examine them further for the presence of T-cell functions such as helper, suppressor and cytotoxic activity. It is apparent that the expression of any of these functions by clonal populations of continuous cell lines would greatly facilitate the analysis of these T cell-mediated functions.

This work was supported by research grants from the National Institutes of Health (grant nos. AI-08917, CA-04681 and HD-01287).

We thank Elaine Bohrer for technical assistance, and Lee and Berri Herzenberg for help in preparation of the manuscript.

RICHARD A. GOLDSBY

NASA-Ames Research Center,
Mountain View, California

BARBARA A. OSBORNE

Department of Genetics,
Stanford University School of Medicine

ELIZABETH SIMPSON

Clinical Research Centre,
Transplantation Biology Section,
Harrow, Middlesex, UK

LEONARD A. HERZENBERG

Department of Genetics,
Stanford University School of Medicine,
Stanford, California, 94305

¹ Köhler, G. & Milstein, C. *Nature* 256, 495-497 (1975).

² Köhler, G. & Milstein, C. *Eur. J. Immun.* 6, 511-519 (1975).

³ Köhler, G., Pearson, T. & Milstein, C. *Somatic Cell Genet.* (in the press).

⁴ Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* 266, 550-552 (1977).

⁵ Krishan, A. *J. Cell Biology* 66, 188-193 (1975).

⁶ Cantor, H. & Boyse, E. A. *Med. Exp.* 141, 1376-1389 (1976).

⁷ Okumura, K., Herzenberg, L. A., Murphy, D., McDevitt, H. & Herzenberg, L. A. *J. Exp. Med.* 144, 685-698 (1976).

The adjuvant active fraction of delipidated mycobacteria

FREUND'S complete adjuvant (FCA) has been used to influence both cell-mediated and humoral immune responses. Mycobacteria are the essential constituents of FCA and different active fractions have been extracted from mycobacteria of various strains¹⁻⁶. The purpose of the work reported here was to try to answer the following question: are cell-mediated and humoral immune responses related to the stimulatory effect of different bacterial components or to the adjuvant activity of a same bacterial fraction? Data presented here suggest that the stimulation of these two kinds of immunological response is related to the adjuvant activity of the same bacterial fraction, which has been identified as the lipoglycopeptide fraction extracted from mycobacterial delipidated cells (LG). Furthermore, the study of the relationship between adjuvant activity and chemical structure showed that LG is comprised of two distinct moieties: a glycopeptide moiety with cellular activity and a lipid moiety stimulating humoral immune response.

LG was isolated, by selective solubilisations, from bacterial products of the human 'Peurois' strain of *Mycobacterium tuberculosis* (Pasteur Institute, Paris) according to the procedure previously described⁷. Cleavage by alkaline hydrolysis⁸ of the ester linkage of LG resulted in two moieties: (1) a liposoluble moiety (37%) mainly composed of unsaturated fatty acids (mycolic acids) as determined by elementary analysis, solubility tests and comparison with the infrared spectrum of an authentic mycolic acid specimen; and (2) a hydrosoluble moiety (63%) identified by paper chromatography and infrared spectrometry, as a glycopeptide. This glycopeptide has the following characteristics: approximate molecular weight of 27,000 (determined by analytical ultracentrifugation); $[\alpha]_D^{20} = 36^\circ$; C=0.1; neutral sugars: 94-98% arabinose and galactose in an approximate molar ratio of 1.6:2; amino acids and amino sugar: 2-6% glycine, alanine, glutamic acid, diaminopimelic acid and galactosamine. Catalytic hydrogenation⁹ of LG resulted in a derivative in which only unsaturated mycolic acids of the lipid moiety were saturated, called lipid moiety modified

derivative (LMD) or glycopeptide moiety non-modified derivative (GND) and acetylation of LG resulted in a derivative in which only free hydroxyl groups of the glycopeptide moiety were acetylated, called glycopeptide moiety modified derivative (GMD) or lipid moiety non-modified derivative (LND).

When LG was submitted to both catalytic hydrogenation and acetylation, a two-moieties-modified derivative was obtained, called LGMD (lipid and glycopeptide moieties modified derivative).

Female albino guinea pigs (400–500 g) were sensitised by injection into each hind footpad of 0.1 ml of a water-in-oil emulsion consisting of 1 part paraffin oil containing the adjuvant fraction, 2 parts Tween 80 and 5 parts saline containing the protein antigen, crystalline ovalbumin (OA). On day 21 after sensitisation, the animals were skin tested by intradermal injection of various amounts of OA in 0.1 ml saline into the clipped flank and the reactions were read 24 and 48 h later.

BD F₁ (DBA/2 × C57BL/6) mice weighing approximately 20 g were each injected intraperitoneally with 10⁸ sheep red blood cells (SRBC) after about 15 min, various amounts of adjuvants in 0.1 ml of the water-in-oil emulsion were given by intraperitoneal injection. After 4 d, the animals were killed and the number of spleen cells forming or releasing antibody to SRBC was determined by the haemolytic plaque assay of Jerne and Nordin¹⁰, without modification.

Figure 1 illustrates the influence of LG and LND on the average number of plaque-forming cells (PFC) in mice 4 d after immunisation with SRBC. No positive reaction was observed in animals which had received LMD or LGMD as adjuvants.

Table 1 shows that inclusion of LG or GND in the antigen injection resulted in strong skin reactivity of delayed type hypersensitivity to the test antigen. No visible skin reaction was observed in animals sensitised with GMD or LGMD.

The results reported here show that in FCA, LG can substitute for whole mycobacterial cells. LG comprises two moieties: a hydrosoluble glycopeptide moiety and a liposoluble lipid moiety. The glycopeptide moiety is responsible

Table 1 Mean diameter of redness in guinea pigs skin at sites of injection of various amounts of test antigen

No. of animals in groups	Sensitising antigen (1 mg per animal)	Sensitisation Adjuvant incorporated in antigen (0.4 mg per animal)	Delayed skin reactions		
			Test antigen dose (mg per 0.1 ml)		
			0.1 mg	3 µg	0.2 µg
6	OA*	FCA	20 (N)†	9	6.5
6	OA*	LG	19 (N)	7	3
6	OA*	GND	20.7 (N)	12	7
6	OA*	GMD	0	0	0
6	OA*	LGMD	0	0	0
6	OA*	None	0	0	0

*Crystalline ovalbumin.

†Mean diameter of redness (mm) at 48 h; N, central necrosis.

FCA, Freund complete adjuvant; GND, glycopeptide moiety non-modified derivative; GMD, glycopeptide moiety modified derivative; LGMD, lipid and glycopeptide moieties modified derivative.

for the cell-mediated response, and the humoral immune response is attributed to the lipid moiety. The ability of LG to act as an adjuvant of both cell-mediated and humoral immune responses seems to be related to some state of balance between these two moieties, since (1) a decrease in hydrosolubility of the glycopeptide moiety by acylation of free hydroxyl groups destroyed cellular activity of LG; (2) an increase in liposolubility of the lipid moiety by catalytic hydrogenation inhibited humoral immune response; and (3) the derivative LGMD in which both lipid and glycopeptide moieties were modified, was found to be completely inactive as an adjuvant for either cell-mediated or humoral immune responses.

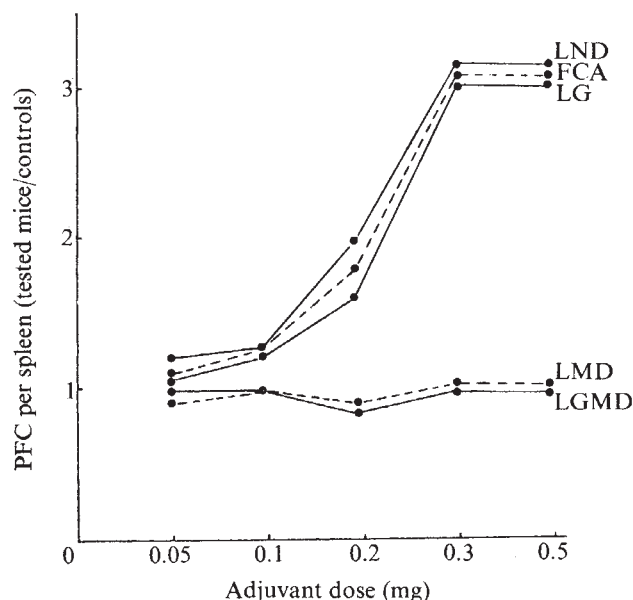
I. J. Hiu

Institut de cancérologie et d'immunogénétique, CNRS, Hôpital Paul-Brousse, 14 Avenue Paul-vaillant-couturier, 94-Villejuif, France

Received 2 March; accepted 27 April 1977.

- Hiu, I. J. *Nature new Biol.* **238**, 241–242 (1972).
- Adam, A., Ciorbaru, R., Pettit, J. F. & Lederer, E. *Proc. natn. Acad. Sci. U.S.A.* **69**, 851–854 (1972).
- Migliore-Samouir, D. & Jolles, P. *FEBS Lett.* **25**, 301–304 (1972).
- Fleck, J., Mock, M., Tytgat, F., Nauciel, C. & Minck, R. *Nature* **250**, 517–518 (1974).
- Stewart-Tull, D. E. S. *et al. Immunology* **29**, 1–15 (1975).
- Chedid, L., Parant, M., Parant, F., Gustafson, R. H. & Berger, F. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 855–858 (1972).
- Hiu, I. J. *Experientia* **31**, 983–984 (1975).
- Hiu, I. J. & Amiel, J. L. *Eur. J. clin. biol. Res.* **16**, 55–58 (1971).
- Hiu, I. J. & Amiel, J. L. *J. gen. Microbiol.* **66**, 239–241 (1971).
- Jerne, N. K. & Nordin, A. A. *Science* **140**, 405 (1963).

Fig. 1 Influence of various amounts of LND, FCA and LG on the average number of direct (IgM) plaque-forming cells (PFC) in mice 4 d after immunisation with sheep red blood cells (SRBC). LG, lipoglycopeptide; LND, lipid moiety non-modified derivative; FCA, Freund's complete adjuvant; LMD, lipid moiety modified derivative; LGMD, lipid and glycopeptide moieties modified derivative.



IgM-IgG switch-over among antibody-forming cells in the mouse

DIFFERENTIATION of immunocytes from stem cells to antibody secreting B cells involves sequential expression of genes coding for heavy chain constant regions. This differentiation seems to be independent of thymus activity and antigen stimulation, for nude mice¹ and 15-week human foetuses² as well as germ-free mice³ and piglets⁴ have proportions of surface IgM, IgG or IgA bearing cells similar to those found in normal adults. It has therefore been suggested that the switch-over from IgM to IgG and IgA expression occurs before antigen contact which leads to differentiation from small B lymphocytes to plasma cells⁵. But during antibody production after antigenic stimulation a small proportion (1–3%) of mouse plaque-forming cells (PFC) secrete both IgM and IgG (refs 6, 7) and clones of cells derived from a single antigen-stimulated precursor secrete both IgM and IgG antibody⁸. These observations indicate that the IgM→IgG switch could be antigen driven. We have investigated the two alternatives by culturing

individual PFC from mice immunised with sheep erythrocytes (SRBC) to study whether daughter PFC produce the same or a different Ig class. We have found that a part of IgM producing parental PFC can generate 'in vitro' IgG

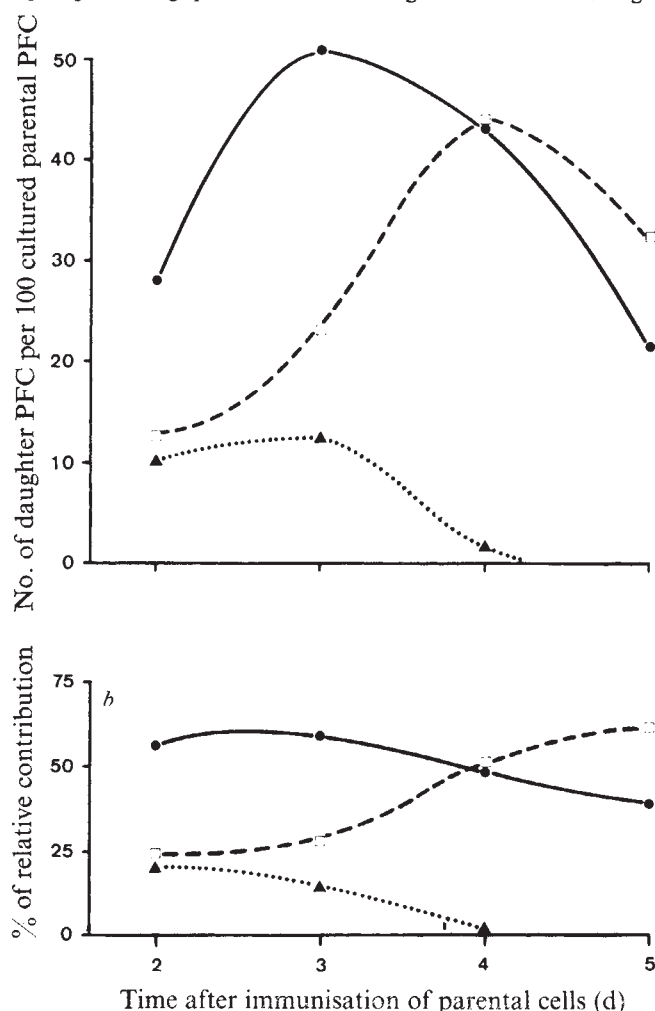


Fig. 1 Generation of daughter PFC producing either IgM or IgG from individually cultured parental PFC taken on the indicated days after immunisation. ●, Parental IgM PFC generating IgM daughters; □, parental IgG generating IgG daughters; ▲, parental IgM generating IgG daughters. *a*, The numbers of daughter PFC are recorded per 100 individually cultured parental PFC (standardised % from 96 to 186 parental PFC at each point). *b*, Relative contribution of various daughter PFC in the total amount of daughter PFC in each day after immunisation. Spleen cells from Swiss mice given SRBC (4×10^8) on day 0 were taken on days 2, 3, 4 or 5 and placed in non-shaken mass cultures as described previously⁹ for selecting the most resistant PFC in 'in vitro' conditions. These cultures were supplemented with LPS at a final concentration of $7.5 \mu\text{g ml}^{-1}$. The cells were taken 24 h later, mixed with SRBC and complement, distributed in 5- μl microdroplets covered with paraffin oil and incubated for 45 min at 37°C . Individual PFC (parental PFC) were micro-manipulated out of the droplets and transferred into separate wells of polyacrylamide plates^{10, 11}. These wells had each been preseeded with about 1,000 heavily irradiated (2,500 rad) normal Swiss mouse spleen cells and 1,000 SRBC. The whole plate was sunk into plastic Petri dishes (Falcon) containing medium made half with the medium taken from the preseeded culture and half with fresh medium. The plates were maintained for 24 h in cell culture conditions and the content of each well was spread together with indicator SRBC and complement on antibody slides (Bellco) incubated for 1 h at 37°C and examined for haemolytic plaque formation (daughter PFC). For investigating the Ig class switch over from the parental to daughter PFC, the parental and daughter PFC were detected either directly (IgM producers) or indirectly using as enhancing serum a rabbit anti-mouse IgG serum mixed with a goat anti-mouse- μ -chain serum for revealing only IgG producing PFC. Control wells containing irradiated cells and SRBC as well as old and fresh culture fluids but not micromanipulated PFC were left in each experimental plate. In a total of over 350 such wells not all PFC were detected.

producing daughters, but this switch occurs only during the first 3 d after immunisation.

Adult mice were immunised with SRBC, their spleens were taken in the following days and cultured for 24 h in mass-cultures and then the PFC were micromanipulated and individually cultured for the subsequent 24 h in the conditions described in Fig. 1. The cultures contained LPS (*Escherichia coli* 055B5 lipopolysaccharide, Difco) for mitogenic stimulation in absence of T-cell helper activity in the individual PFC cultures. Three series of experiments were undertaken: in the first IgM producing (direct) parental PFC appearing in the mass-cultures were individually cultured and then plated with the direct procedure for revealing IgM producing daughter PFC; in the second series, the parental and daughter PFC were revealed with the indirect procedure using an enhancing an anti-mouse IgG serum mixed with an anti-mouse μ -chain serum (Table 1); in the third series, again IgM producing parental PFC were individually cultured but the daughter PFC were revealed by the indirect method for detecting only IgG producers so as to study the IgM→IgG switch from parental to daughter PFC.

In this type of experiment not all individually cultured PFC yield a progeny. In fact, only 10–20% of parental PFC generate daughter PFC (refs 11, 13). But, the percentage of parental PFC yielding daughter PFC and especially the number of the latter (1–5 per parental PFC), can give a representative figure of the proliferative potential of each category of parental PFC. As shown in Fig. 1*a* day 3 parental IgM PFC generated the higher number of IgM daughters (51%) and thereafter their yield decreased whereas the 4th day IgG parents produced the higher number of IgG daughters (44%). The generation of IgG producing daughter cells from day 2 and day 3 parental IgM producers was rather low (10% and 12.5% respectively) and sharply decreased thereafter (1.5% from day 4 and 0% from day 5 parental IgM PFC). It was, however, significant because the relative contribution of IgG daughters of IgM PFC seems quite considerable, about the half on day 2 and the third on day 3 in the total number of IgG producing daughters of IgG and IgM parental PFC (Fig. 1*b*). On the other hand, it seems that not all IgM PFC can generate IgG daughters: 21% of day 3 parental IgM PFC (39 out of 186) produced IgM daughters (total, 95; 51%) whereas only 8.7% (14 out of 160) of day 3 parental IgM PFC generated IgG daughters (total 20; 12.5%). This is in agreement with observation that a proportion of lymphocytes bear surface IgM only whereas others express both IgM and IgG or IgM and IgA before expressing the latter alone¹. The low numbers of IgG daughters of IgM PFC detected may be due to the generation of both IgM and IgG daughters from these IgM parental PFC. It is also likely that these parental PFC already began to synthesise low quantities of IgG. These possibilities could not be tested using our methods because parental and daughter PFC endowed with high rate synthesis and secretion of Ig could only be revealed by either the direct or the indirect method.

To what extent do the above findings correspond to the 'in vivo' events? To answer this question we compared the relative frequency of IgM and IgG producing daughter PFC with that of IgM and IgG PFC found in 'in vivo' studies. Wortis *et al.*¹⁴ observed that on the 3rd day after immunisation of mice with SRBC, 47% of PFC produced IgM and 53% IgG and on the 5th day 38% and 62% respectively. Sell *et al.*¹⁵ did not find significant numbers of IgG PFC on day 3 whereas on day 5 they found 42% IgM PFC and 58% IgG PFC. Compared with these figures we recorded 59% IgM and 41% IgG daughters of day 3 parental PFC and 39% and 61% respectively from day 5 parental PFC (Fig. 1*b*). Therefore it could be inferred that this sampling of daughter PFC is not very different from that found 'in vivo' at correspond-

Table 1 Numbers of haemolytic PFCpcr 10^6 spleen cells taken on day 2 or 5 after immunisation of mice with SRBC

Days after immunisation	Direct PFC	Anti- μ chain 1 : 200	PFC enhanced with Anti-IgG 1 : 3, 200*	Anti- μ 1 : 200 + anti-IgG 1 : 3, 200†
2	98	2	122	6
	48	1	57	2
	36	0	43	3
	60	2	78	4
5	796	3	1,120	496
	687	0	2,374	415
	835	2	1,608	1,083
	1,078	5	2,530	885

*Rabbit anti-mouse IgG serum (Pasteur Institute, Paris) exhibiting its maximum enhancing activity at the dilution of 1.3,200.

†Goat anti-mouse IgM serum (Meloy) made monospecific anti- μ -chain by absorption with mouse IgG in the laboratory and giving in immunoelectrophoresis a single arc of precipitation corresponding to mouse IgM (ref. 12).

ing intervals after immunisation with SRBC. This parallelism does not exclude the possibility that the IgM→IgG switch described here is induced by LPS present in the cultures since it has been shown the LPS alone accelerate expression of surface and cytoplasmic IgG¹⁶. Secretion of IgG 'in vitro' does not appear until 2 weeks after onset of LPS stimulated mouse spleen cell cultures¹⁷, however.

The considerable frequency of IgG PFC daughters of day 2 IgM PFC (44% of total IgG daughters, Fig. 1b) may mean that on day 0 of primary stimulation only IgM precursors are triggered by antigen. The more so as treatment of cells with anti-mouse μ -chain serum when culturing them¹⁸ or transferring them into irradiated recipients¹⁹ results in inhibition of IgM as well of IgG and IgA antibody production. Cells already committed to IgG could also be triggered by antigen during primary (Fig. 1) or during secondary response²⁰, however and generate progeny of IgG producing cells. It is therefore possible that even during the primary response a part of IgG producers are derived from IgG precursors.

Lawton *et al.*⁵ studying the development of Ig class diversity concluded that the switch from IgM to IgG expression occurs before antigen contact. Our results showing that early after antigen stimulation a part of IgM PFC generate IgG producers suggest that the cells are sensitive to antigen before the full accomplishment of the commitment to one particular Ig class production. The process of sequential expression of genes coding for C_H could, however, be independent of the antigen-driven differentiation from B lymphocytes to plasma cells. Indeed findings that germ-free mice³ and piglets⁴ have normal adult proportions of cells bearing μ , γ or α surface Ig as well as present evidence that on days 4 and 5 after immunisation no IgM→IgG switch occurs in spite of the existence of many proliferating IgM PFC and the presence of antigen (Fig. 1) strongly suggest that IgM→IgG switch is rather directed by an internal genetic control. It may in fact be that B cells become sensitive to antigen as early as Ig (IgM) molecules appear on their surface, the switch-over to IgG or IgA production taking place whether or not antigenic stimulation intervenes.

This work was supported by the CNRS (grant RCP290), France.

C. BLEUX
M. VENTURA
P. LIACOPOULOS

Institut d'Immuno-Biologie
Hôpital Broussais
96 rue Didot
75674 Paris Cedex 14, France

Received 21 February; accepted 28 April 1977.

¹ Bankhurst, A. D. & Warner, N. L. *Austr. J. exp. Biol. med. Sci.* 50, 661–664 (1972).

² Cooper, M. D., Keightley, R. G., Wu, L. Y. F. & Lawton, A. R. *Transpl. Rev.* 16, 51–84 (1973).

³ Lawton, A. R. & Cooper, M. D. in *Contemp. Top. Immunobiol.* 3, 193–225 (1974).

⁴ Binns, R. M., Feinstein, A., Gurner, B. W. & Coombs, R. R. A. *Nature new Biol.* 239, 114 (1972).

⁵ Lawton, A. R., Kincade, R. W. & Cooper, M. D. *Fedn Proc.* 34, 33–39 (1975).

⁶ Ivanyi, J. & Dresser, W. *Clin. exp. Immun.* 6, 493–501 (1970).

⁷ Nossal, G. V., Warner, N. & Lewis, H. *Cell. Immun.* 2, 41–53 (1971).

⁸ Press, J. L. & Kliman, N. R. *J. exp. Med.* 118, 300–305 (1973).

⁹ Couderc, J., Bleux, C., Birrien, J. L. & Liacopoulos, P. *Immunology* 29, 653–664 (1975).

¹⁰ Marbrook, J. & Haskill, J. S. *Cell. Immun.* 13, 12–21 (1974).

¹¹ Cunningham, A. J. & Fordham, S. A. *Nature* 250, 669 (1974).

¹² Couderc, J., Birrien, J. L., Oriol, R., Bleux, C. & Liacopoulos, P. *Eur. J. Immun.* 5, 140–147 (1975).

¹³ Liacopoulos, P., Couderc, J. & Bleux, C., *Ann. Immun. Inst. Pasteur* 127C, 519–530 (1976).

¹⁴ Wortis, H. H., Dresser, D. W. & Anderson, H. R. *Immunology* 17, 93–110 (1969).

¹⁵ Sell, S., Park, A. B. & Nordin, A. A. *J. Immun.* 104, 483–494 (1970).

¹⁶ Kearney, J. F., Cooper, M. D. & Lawton, A. R. *J. Immun.* 117, 1567–1572 (1976).

¹⁷ Zauderer, M. & Askonas, B. A. *Nature* 260, 611–613 (1976).

¹⁸ Pierce, C. W., Soliday, S. M. & Asofsky, R. J. *exp. Med.* 135, 675–697 (1972).

¹⁹ Herrod, H. G. & Warner, N. L. *J. Immun.* 108, 1712–1717 (1972).

²⁰ Mason, D. W. & Gowans, J. L. *Annals Immunol. Inst. Pasteur* 137C, 657–666 (1976).

Simple model for self-non-self-discrimination in invertebrates

THE immune mechanisms which enable invertebrates to eliminate infectious agents are not well understood. There have been many reports, however, that invertebrates produce 'recognition factors' which interact with foreign substances and subsequently aid the phagocytosis of these substances^{1–5}. Such recognition factors have been isolated from worms, molluscs, arthropods, echinoderms and protochordates, and have been shown to have agglutinating and bactericidal activities as well as opsonic properties. But, unlike the vertebrate immune system, there is little evidence for a memory component in the production of these factors^{3–5}. The recognition factors are usually large molecules which can be readily dissociated into subunits and which in no way resemble the immunoglobulins present in vertebrates^{3–12}. They apparently have specificity for carbohydrate structures and, in many cases, an individual has several distinct subpopulations of recognition factors which bind different sugar specificities^{3–12}. The recognition factors seem to be synthesised by haemocytes in the haemolymph^{13,14}. After secretion they can, in many invertebrate species, bind to the surface of haemocytes as well as persist in a soluble form in the haemolymph^{3–5,14–18}. Both the soluble and cell-bound forms of the recognition factors can augment the phagocytosis of foreign substances. Thus, the invertebrates have evolved a simple recognition system which enables them to discriminate between 'self' and 'non-self'. In this paper I propose that the recognition factors are composed of some of the glycosyl transferases which the invertebrate uses to synthesise its own carbohydrate side chains. Such a model guarantees the maintenance of rigid self non-self discrimination.

The model is presented schematically in Fig. 1 and would particularly apply to the advanced invertebrates. It is arbitrarily proposed that the invertebrate synthesises an oligosaccharide side chain of five sugar residues. Each sugar residue is attached by a different glycosyl transferase which has specificity for the sugar acceptor¹⁹. Thus, five proteins (transferases) with different sugar binding properties are

present in the organism. These five transferases act as the subunits of the recognition factors, are secreted by haemocytes into the haemolymph, and randomly associate into hexamers.

The recognition factors do not react against self as the self sugars they recognise have been blocked by glycosylation. This important point is made clearer if the mode of action of glycosyl transferases is considered (Fig. 2). These enzymes catalyse the transfer of a monosaccharide from a sugar nucleotide to the terminus of a specific sugar acceptor¹⁹. Figure 2 depicts the enzyme-substrate reaction of glycosyl transferase 4 in Fig. 1. It can be seen that transferase 4 interacts with terminal monosaccharide 3 and catalyses the attachment of monosaccharide 4. Of course, once glycosylation has occurred, monosaccharide 3 is blocked from further interaction with transferase 4. Thus, the glycosyl transferases which make up the recognition factors are unable to bind to self as the terminal sugars they recognise have been masked by glycosylation. In addition, it should be noted that the recognition factors would probably only bind foreign substances and not glycosylate them (as in Fig. 2) as it is most unlikely that sugar nucleotides exist extracellularly in the haemolymph.

In the model presented in Fig. 2, it is proposed that polymerisation of the transferases into a recognition factor is initiated by an additional protein (no. 6) which has an acceptor site on the haemocyte surface. The inclusion of this additional protein imparts cytophilic and opsonic properties to the recognition factors. In order to prevent inter-

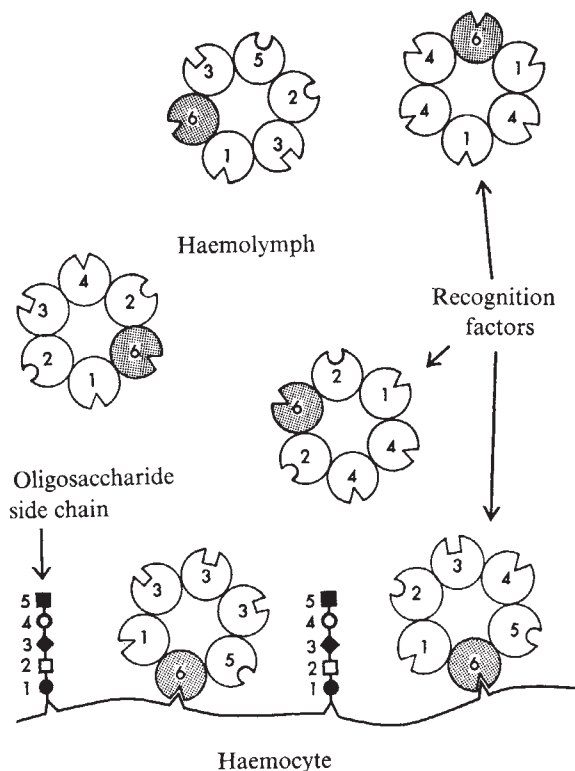
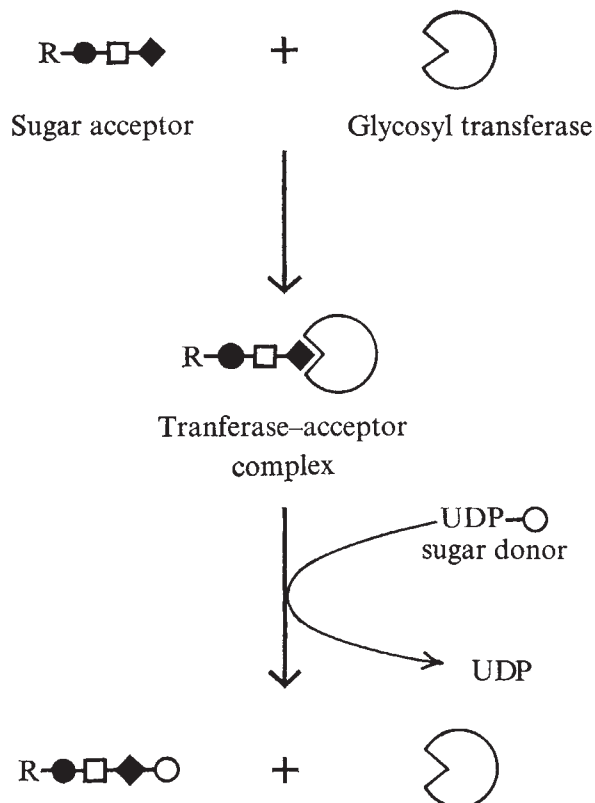


Fig. 1 A possible model for the recognition factors in invertebrates which augment the phagocytosis of foreign substances. Various monosaccharides on the haemocyte surface, which constitute an oligosaccharide side chain, are represented by circles, diamonds and squares and are numbered 1-5. Each monosaccharide is inserted in the oligosaccharide by a specific glycosyl transferase (proteins numbered 1-5) as depicted in Fig. 2. Thus, transferase 2 binds to monosaccharide 1 and catalyses the attachment of monosaccharide 2, transferase 3 binds to monosaccharide 2 and facilitates the attachment of monosaccharide 3, and so on. The transferases randomly associate into recognition factors (hexamers), this polymerisation being initiated by an additional protein (no. 6). Protein 6 has an acceptor site on the haemocyte surface which enables the recognition factors to be cytophilic for haemocytes. For details of the model see text.



Reaction product

Fig. 2 Mode of action of glycosyl transferases. A trisaccharide sugar acceptor is depicted, linked to a lipid or protein carrier (R). The circles, diamonds and squares represent the different monosaccharides. The glycosyl transferase specifically binds to the terminal monosaccharide (◆) of the sugar acceptor and catalyses the transfer of a monosaccharide (○) from its uridine diphosphate (UDP) donor to the terminus of the acceptor. The resultant reaction product is a tetrasaccharide. This diagram depicts the enzyme-substrate reaction of glycosyl transferase (no. 4) in Fig. 1, that is, the transferase which binds to monosaccharide 3 and catalyses the attachment of monosaccharide 4.

action (and therefore autoaggression) between haemocytes, only one cytophilic subunit can be included in each hexamer.

Although there are only five basic recognition units in this model, the fact that they polymerise in a random manner into hexamers, enables them to react in a multi-point fashion with foreign substances. This greatly increases the effective specificity range of the recognition factors as it is well established that multipoint interactions have a much higher binding efficiency than single point interactions²⁰.

This model of immune recognition has several important theoretical advantages. First, it ensures that self-reactive recognition factors are not generated. Second, due to multipoint binding, a comparatively wide range of foreign antigenic specificities can be recognised. Third, the system can withstand a high degree of genetic polymorphism without self-reactivity appearing. Fourth, if any infectious agent produces hydrolytic enzymes which degrade the cell surface carbohydrates of the invertebrates host, the subterminal sugars are unmasked and thus these damaged and infected cells can be readily recognised and encapsulated by the host's haemocytes.

The model presented in Fig. 1, however, could vary in several ways. Obviously, the variety of glycosyl transferases and the number of subunits incorporated into the recognition factors could vary considerably between invertebrate species. Furthermore, the cytophilic subunit may be absent in some invertebrates and, in these cases, the factors may eliminate infectious organisms by merely acting

as lytic and neutralising agents. Similarly, only some of the glycosyl transferases produced by the invertebrate may be secreted and incorporated into recognition structures. Conversely, not all the glycosyl transferases appearing in the recognition factors may be able to act as glycosylating enzymes. For example, if a mutant form of enzyme 3 arose (see Fig. 1) which had lost its glycosylating activity, then enzymes 4 and 5 would be unable to construct a sugar side chain, even though their enzymatic properties were still intact.

There would be no memory component in this form of immune recognition. It is conceivable, however, that infectious agents may activate the haemocytes to multiply and secrete the recognition factors more rapidly. There is, in fact, some evidence in invertebrates that foreign substances can induce the production of recognition factors^{3,5,21,22}. Whether the recognition factors described above mediate the specific transplantation immunity observed in some invertebrate species remains to be determined^{3,5,23,24}. The existence of specific short-term memory in these transplant responses suggests, however, that another recognition system, possibly the forerunner of vertebrate T-cell immunoglobulin, may be involved²⁵.

In conclusion, it should be noted that there is a mounting body of evidence which suggests that, in both plants and animals, glycosyl transferases have a role in cell-cell recognition and cell-glycoprotein interactions^{19,26,27}. These findings add credence to the possibility that glycosyl transferases could have developed into a primitive protective system against infectious agents. In fact, some of the sugar binding proteins detected in vertebrates, such as the galactose-binding proteins in liver which mediate the clearance of desialysed plasma proteins^{26,28,29}, may represent a remnant of the invertebrate recognition system described.

C. R. PARISH

Department of Microbiology,
John Curtin School of Medical Research,
Australian National University,
Canberra, A.C.T. Australia, 2601

Received 7 March; accepted 25 April 1977.

- 1 Tripp, M. R. *J. Invert. Path.* **8**, 478–488 (1966).
- 2 McKay, D., Jenkin, C. R. & Rowley, D. *Aust. J. exp. Biol. med. Sci.* **47**, 125–134 (1969).
- 3 Hildemann, W. H. & Reddy, A. L. *Fedn Proc.* **32**, 2188–2194 (1973).
- 4 Cohen, E. & Uhlenbruck, G. (eds) *Ann. N.Y. Acad. Sci.* **234**, (1974).
- 5 Cooper, E. L. (ed.) *Contemp. Topics Immunobiol.* **4**, (Plenum, New York, 1974).
- 6 Cohen, E. *Trans. N.Y. Acad. Sci.* **30**, 427–443 (1968).
- 7 Marchalonis, J. J. & Edelman, G. M. *J. molec. Biol.* **32**, 453–465 (1968).
- 8 Acton, R. T., Bennett, J. C., Evans, E. E. & Schrohenloher, R. E. *J. biol. Chem.* **244**, 4128–4135 (1969).
- 9 Pauley, G. B., Granger, G. A. & Krassner, S. M. *J. Invert. Pathol.* **18**, 207–218 (1971).
- 10 Jenkin, C. R. & Hardy, D. *Adv. exp. Med. Biol.* **64**, 55–65 (1975).
- 11 Hall, J. L. & Rowlands, D. T. *Biochemistry* **13**, 821–827 (1974).
- 12 Hall, J. L. & Rowlands, D. T. *Biochemistry* **13**, 828–832 (1974).
- 13 Marck, M. *Comp. Biochem. Physiol.* **35**, 615–622 (1970).
- 14 Amiante, G. A. *Experientia* **32**, 526–528 (1976).
- 15 Scott, M. T. *Immunology* **21**, 817–828 (1971).
- 16 Tyson, C. J. & Jenkin, C. R. *Aust. J. exp. Biol. Med. Sci.* **52**, 341–348 (1974).
- 17 Renwrantz, L. R. & Cheng, T. C. *J. Invert. Pathol.* **29**, 88–96 (1977).
- 18 Renwrantz, L. R. & Cheng, T. C. *J. Invert. Pathol.* **29**, 97–100 (1977).
- 19 Roseman, S. *Chem. Phys. Lipids* **5**, 270–297 (1970).
- 20 Karush, F. *Ann. N.Y. Acad. Sci.* **169**, 56–62 (1970).
- 21 Evans, E. E. *et al. Nature* **222**, 696–697 (1969).
- 22 Evans, E. E., Painter, B., Evans, M. L., Weinheimer, P. & Acton, R. T. *Proc. Soc. exp. Biol. Med.* **128**, 394–398 (1968).
- 23 Cooper, E. L. *Transplant. Proc.* **2**, 216–221 (1970).
- 24 Duprat, P. C. *Transplant. Proc.* **2**, 222–225 (1970).
- 25 Binz, H. & Wigzell, H. *Scand. J. Immun.* **5**, 559–571 (1976).
- 26 Shur, B. D. & Roth, S. *Biochim. biophys. Acta.* **415**, 473–512 (1975).
- 27 Albersheim, P. & Anderson-Prouty, A. J. *A. Rev. Pl. Physiol.* **26**, 31–52 (1975).
- 28 Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, J. & Ashwell, G. *J. biol. Chem.* **246**, 1461–1467 (1971).
- 29 Hudgin, R. & Ashwell, G. *J. biol. Chem.* **249**, 7369–7372 (1974).

Succinyl bee venom melittin is a leukocyte chemotactic factor

A NUMBER of leukocyte chemotactic factors of defined structure have been described. They include formyl-methionyl tripeptides (f-Met-Leu-Phe and related peptides) which attract neutrophils at concentrations as low as 10^{10} – 10^{11} M (refs 1 and 2), the eosinophilotactic tetrapeptides Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu (ECF-A) (ref. 3) and

HETE; a 12-hydroxy-unsaturated fatty acid derivative of arachidonic acid⁴. All of these have in common a net negative charge and a hydrophobic moiety. The availability of chemotactically active molecules of known structure now allows structure-activity relationships to be examined, and preliminary evidence that f-Met peptides bind to a receptor on the leukocyte surface has been presented^{5,6}. Leukocytes respond to a great many chemotactic factors of diverse structure and, in order to understand fully the binding sites involved, it will be useful to have as many defined chemotactic factors for study as possible. This paper reports the chemotactic activity of the succinylated form of the bee venom peptide, melittin.

Figure 1 shows the structure of melittin⁷. It is a cationic, amphipathic peptide in which residues 1–20 are predominantly hydrophobic and residues 21–26 hydrophilic. Melittin is surface-active and has been shown to disrupt cell membranes and artificial phospholipid liposomes^{8,9}. Unmodified melittin is lytic for neutrophil leukocytes at concentrations of 10^{-6} M and above⁸, and I was unable to detect any chemotactic activity for unmodified melittin at any concentration. Habermann and Krowallek¹⁰ showed that the cytolytic effects of melittin could be abolished if the α - and ϵ -amino groups were blocked by succinylation, thus reducing the positive charge. Since succinylation would yield a product with broadly similar physicochemical characteristics to the chemotactic factors listed above, it might be predicted that succinyl-melittin (S-melittin) would have chemotactic activity for leukocytes.

Gly – Ile – Gly – Ala – Val – Leu – Lys – Val – Leu – Thr –
Thr – Gly – Leu – Pro – Ala – Leu – Ile – Ser – Trp – Ile –
Lys – Arg – Lys – Arg – GluNH₂ – Glu(NH₂)₂

Fig. 1 Structure of melittin.

Figure 2 shows that a pure preparation of S-melittin attracts human blood neutrophil leukocytes into micropore filters in a dose-dependent manner. To demonstrate that the cells were responding to a gradient of S-melittin and thus that S-melittin determined the direction of their locomotion (chemotaxis) and not merely their rate of locomotion (chemokinesis), the activity of S-melittin was examined in a checkerboard assay, a procedure introduced by Zigmond and Hirsch¹¹ to distinguish between these two reactions.

Table 1 shows that the leukocytes responded to both the absolute concentration of S-melittin and to the concentration gradient, thus showing both reactions. Assuming that melittin exists as a tetramer⁷, its effective concentration was between 5×10^{-5} M and 5×10^{-6} M. Only heavily succinylated preparations of melittin were sufficiently non-cytotoxic to show chemotactic effects and at least 80% of free amino groups required to be blocked to obtain a chemotactically active peptide.

Melittin contains neither methionine nor phenylalanine, and thus does not contain any sequence similar to those of the f-Met peptides which are active as chemotactic factors. If these peptides act by binding to a stereospecific cell surface receptor, it is unlikely that S-melittin binds to the same receptor. It is more probable that the leukocyte surface contains more than one site at which chemotactic reactions can be initiated. The effective chemotactic concentration of S-melittin was lower (ED_{50} about 5×10^{-8} to 10^{-9} M) than that of f-Met tripeptides suggesting a lower binding affinity. The locomotor response induced by the ED_{50} was, however, comparable in both cases.

Since melittin is known to bind to lipid bilayers both in cells and in liposomes, it is suggested that this binding, which could be to a saturable binding site but is not likely

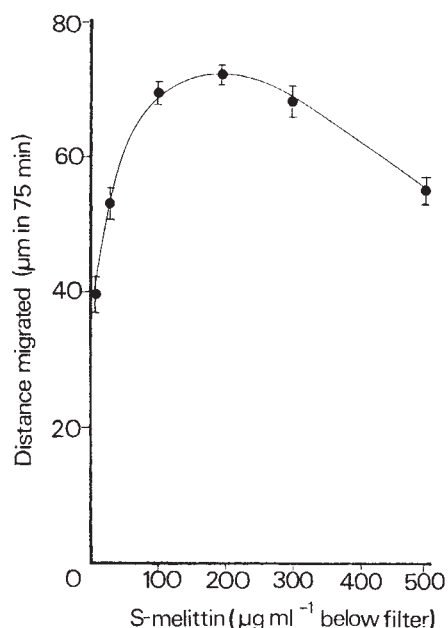


Fig. 2 Migration of human blood neutrophils into micropore filters (3- μ m poresize, Millipore) towards S-melittin at various concentrations below the filter. Cells were prepared in Gey's solution with human serum albumin (1 mg ml⁻¹) and the chemotaxis assay was carried out as described previously¹³ using the leading-front method¹¹ to measure migration. Bee venom melittin was a highly purified product obtained from Dr Barbara Banks, University College, London, UK. It was treated with succinic anhydride (35 mg per 20 mg peptide) as described by Habermann and Krowallek¹⁰. The ninhydrin reaction¹⁴ was used to determine free amino groups before and after succinylation. The preparation used in the experiment shown had 83% of its free amino groups blocked.

to be to a stereospecific receptor, is an adequate signal for initiation of chemotactic reactions. I have presented a similar hypothesis to explain the chemotactic properties of denatured proteins and proteins with diverse covalently bonded non-polar groups¹². It now seems likely that chemotactic reactions can be initiated by signals from molecules binding to the cell surface at a variety of sites and with a variety of affinities. This versatility seems reasonable in view of the wide diversity of molecules now shown to initiate such reactions¹³. The charge of surface-active chemotactic factors such as S-melittin is obviously important as well as their hydrophobicity, since blocking the amino

Table 1 Migration of human blood neutrophils in various absolute concentrations and concentration gradients of S-melittin

S-melittin μg ml ⁻¹	Migration (μm into 3-μm poresize filter in 75 min)				
	0	52	95	185	275
0	52				
5		54	82(62)	89(70)	102(65)
95		58(63)	71		95(73)
185		68(73)		75	
275		57(78)	76(81)		91

Figures on the diagonal from upper left to lower right show migration in increasing absolute concentrations of S-melittin but in the absence of a gradient. Above the diagonal, cells were moving in a positive gradient, below it in a negative one. The figures without brackets show the migration actually observed, those within brackets show the migration expected if the cells had been responding to absolute concentration alone (calculated after Zigmond and Hirsch¹¹). Note that cells migrated further in positive gradients and less far in negative gradients than would be expected if they had responded only to the absolute concentration of the attractant suggesting that there was a chemotactic response to the S-melittin gradient.

groups of melittin with negatively charged succinyl groups yields a product which is chemotactic at concentrations at which the unconjugated, cationic, analogue damages the cell.

P. C. WILKINSON

Department of Bacteriology and Immunology,
University of Glasgow (Western Infirmary),
Glasgow, UK

Received 23 March; accepted 28 April 1977.

- Schiffmann, E., Corcoran, B. A. & Wahl, S. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1059-1062 (1975).
- Showell, H. J. *et al. J. exp. Med.* **143**, 1154-1169 (1976).
- Goetzl, E. J. & Austen, K. F. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4123-4127 (1975).
- Turner, S. R., Tainer, J. A. & Lynn, W. S. *Nature* **257**, 680-681 (1975).
- Becker, E. L. in *Molecular and Biological Aspects of the Acute Allergic Reaction* (eds S. G. O. Johansson, K. Strandberg & B. Uvnäs) 353-370 (Plenum, New York, 1976).
- Schiffmann, E. in *Leukocyte Chemotaxis; Methodology, Physiology, Clinical Implications* (eds J. I. Gallin & P. G. Quie) (Raven, New York, 1977).
- Habermann, E. & Jentsch, J. *Hoppe Seyler's Z. Physiol. Chem.* **348**, 37-50 (1967).
- Weissmann, G., Hirschhorn, R. & Krakauer, K. *Biochem. Pharmac.* **18**, 1771-1775 (1969).
- Sessa, G., Freer, J. H., Colacicco, G. & Weissmann, G. *J. biol. Chem.* **224**, 3575-3582 (1969).
- Habermann, E. & Krowallek, H. *Hoppe Seyler's Z. Physiol. Chem.* **351**, 884-890 (1970).
- Zigmond, S. H. & Hirsch, J. G. *J. exp. Med.* **137**, 387-410 (1973).
- Wilkinson, P. C. *Clin. exp. Immun.* **25**, 355-366 (1976).
- Wilkinson, P. C. *Chemotaxis and Inflammation* (Churchill-Livingstone, Edinburgh, 1974).
- Moore S. & Stein, W. H. *J. biol. Chem.* **211**, 907-913 (1954).

Theophylline-induced changes in ion transport and conductance in human small intestinal mucosa

THE central role of cyclic 3',5'-adenosine monophosphate in intestinal secretion has been emphasised recently^{1,2}. Electrolyte absorption is converted to secretion^{3,4} and mucosal conductance is reduced^{5,6} by increases in epithelial cyclic AMP concentrations and this is held to mediate the profound secretion provoked by cholera and other forms of infective 'toxicogenic' diarrhoea⁷. The mechanisms of the intestinal response to cyclic AMP are incompletely understood and we therefore examined this response in human intestinal mucosa. We report here that changes in mucosal permeability are largely determined by the state of the lateral intercellular spaces. When these spaces are open, permeability is increased by cyclic AMP and when they are closed, permeability is decreased. These changes in permeability have a profound effect on the ion transport responses to cyclic AMP.

In ileal mucosa theophylline induced a rapid rise in electrical potential difference (p.d.) and short circuit current (s.c.c.) persisting for at least 40 min (Fig. 1). Total ionic conductance, calculated from p.d. and s.c.c., fell by about 25%, an effect similar to that observed in rabbit ileum⁸.

This conductance fall has been attributed to effects on paracellular shunt pathways and one possible anatomical counterpart is the elimination of lateral intercellular spaces which follows cell swelling induced by theophylline⁶. This high conductance pathway normally contributes about 80% of total epithelial conductance⁹, and closure of the channels could clearly influence total conductance. If this hypothesis is correct, then opening the spaces should prevent this response to theophylline. We therefore re-examined the influence of theophylline in the presence of glucose (15 mM) which dilated the lateral spaces due to enhanced fluid absorption (Fig. 2). Here, instead of rising, the mucosal resistance, the reciprocal of conductance, fell from 37 to 21 Ω cm⁻² (Fig. 1). 3-O-methyl glucose also caused theophylline to lower ileal resistance from 41 to 26 Ω cm⁻² and 35 to 28 Ω cm⁻² in two studies. Since 3-O-methyl glucose is transported but not metabolised, this response is not a non-

specific metabolic effect of glucose. It also occurred with glucose on the mucosal, but not with it present solely on the serosal side of the tissue, confirming an association with transport. Glucose alone did not influence resistance, but did so when added to tissues pretreated with theophylline (Fig. 1).

The simplest explanation for these findings is that dilatation of lateral spaces revealed a theophylline-induced increase in conductance. This would bring intestinal mucosa into line with high resistance epithelia such as toad bladder, in which theophylline enhances conductance¹⁰. Theophylline thus apparently has two effects—decreasing conductance due to cell swelling and closure of lateral spaces, and increasing it when the lateral spaces are opened by glucose. Similar changes were found in rat ileum by Walling and Kimberg (personal communication), but others have not demonstrated this effect in rabbit ileum exposed to cholera toxin¹¹. This discrepancy may be due to species differences, but is otherwise unexplained.

There is some conflict about the mechanisms for the

intestinal secretion. Nellans *et al.*¹² demonstrated that theophylline inhibits a coupled Na-Cl influx at the luminal cell membrane while Powell *et al.*¹³ presented convincing evidence for the stimulation of a coupled Na-Cl secretion in rabbit ileum. Yet others have postulated that theophylline stimulates a rheogenic secretion of Cl, uncoupled from Na (ref. 14). Since mucosal conductance influences electrolyte transport we examined the effect of theophylline on ion

Fig. 2 Electron micrograph of ileal mucosa after exposure to theophylline in the absence of glucose (a) and in its presence (b). Note the dilated intercellular spaces in the latter.

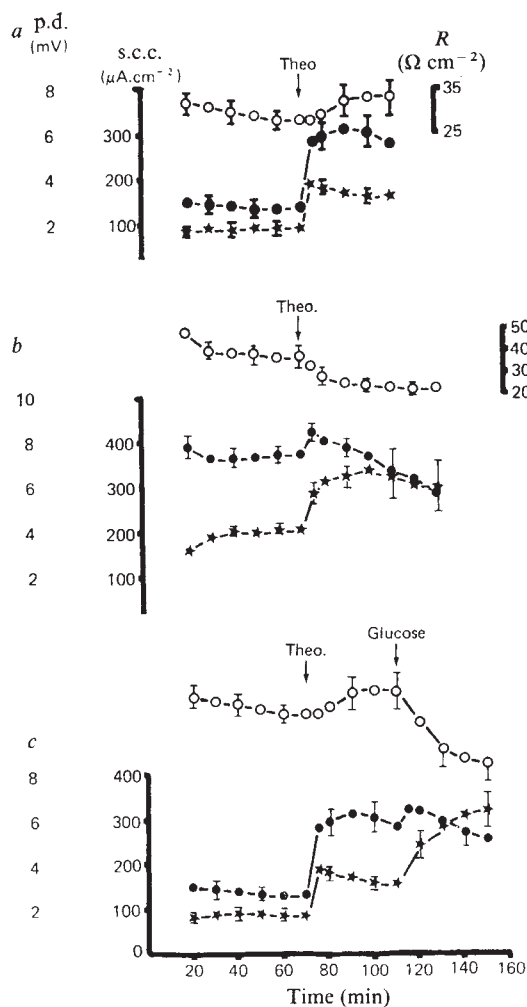
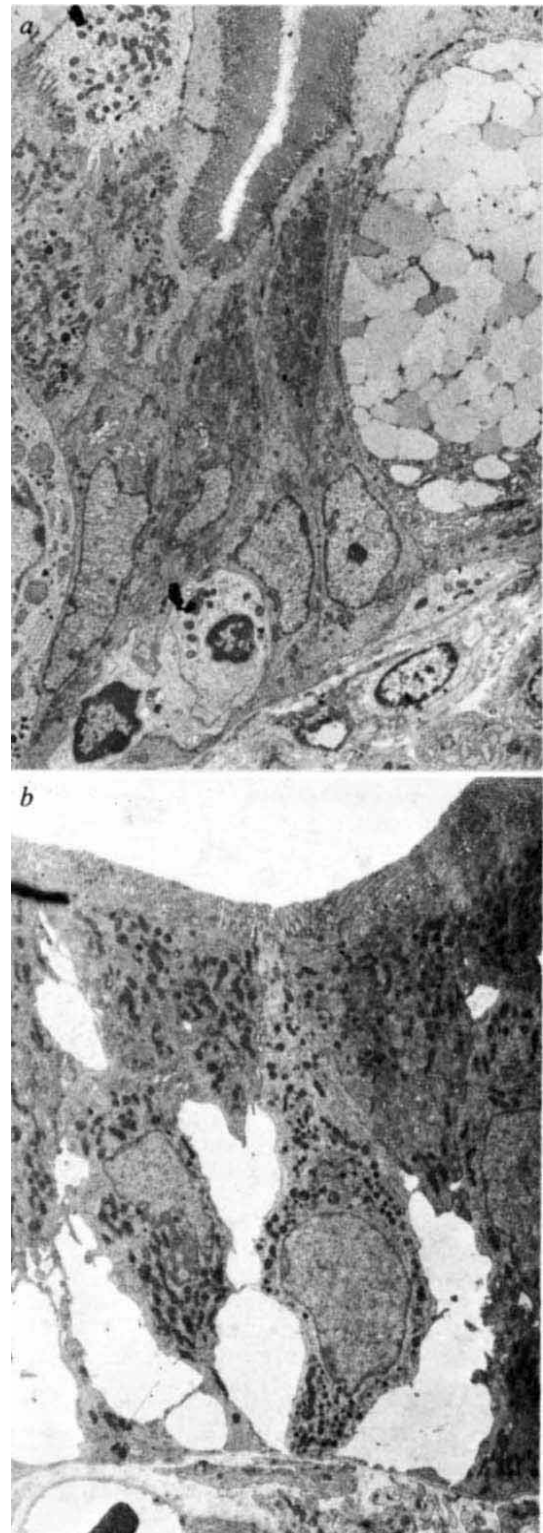


Fig. 1 Electrical responses of stripped human ileal mucosa to theophylline (10^{-2} M). Theophylline (Theo.) raises mucosal cyclic AMP concentrations by inhibiting its breakdown. Fresh mucosa, removed at operation for a variety of diseases, was separated from abnormal areas, stripped of muscle and serosa and mounted in Ussing type flux chambers as described previously⁸. In *a* glucose was absent from the bathing solutions; in *b* it was present throughout (15 mM), and in *c* it was added 40 min after the theophylline. In each case the p.d. (●) and s.c.c. (★) rose in response to theophylline but the calculated tissue resistance (*R*) (○) rose in the absence of glucose and fell in its presence. Glucose alone did not influence resistance but it fell when glucose was added after theophylline (*c*) indicating that this effect is dependent on the presence of both glucose and theophylline.

Table 1 Influence of theophylline (10^{-2} M) on ion transport ($\mu\text{mol cm}^{-2} \text{h}^{-1}$) in stripped ideal mucosa in glucose-free and glucose-containing media and across jejunal mucosa in glucose-containing media

	J Na ms	J Na sm	J Na net	J Cl ms	J Cl sm	J Cl net	J R net	I.s.c.
Ileum								
No glucose ($n = 11$) (control)	11.2 ± 1.0	9.52 ± 0.72	+ 1.68 ± 0.75	9.59 ± 0.98	10.93 ± 0.4	- 1.34* ± 0.71	+ 0.80* ± 0.45	3.82 ± 0.42
Theophylline	9.12 ± 0.70	10.99 ± 0.78	- 1.87 ± 0.89	8.00 ± 1.00	13.68 ± 0.69	- 5.68 ± 0.6	+ 1.94* ± 0.83	5.75 ± 0.69
<i>P</i>	0.05	< 0.005	< 0.01	< 0.05	< 0.001	< 0.001	NS	< 0.005
Glucose ($n = 9$) (control)	20.1 ± 1.9	8.3 ± 0.3	+ 11.8 ± 1.7	12.4 ± 0.9	8.8 ± 0.6	+ 3.6 ± 0.8	+ 05.* ± 1.2	8.7 ± 1.1
Theophylline	20.9 ± 2.6	11.3 ± 1.1	+ 9.6 ± 2.2	10.5 ± 1.3	13.3 ± 0.9	- 2.8 ± 1.2	+ 0.3* ± 2.2	12.7 ± 1.8
<i>P</i>	NS	< 0.02	NS	NS	< 0.01	< 0.01	NS	< 0.01
Jejunum								
Glucose ($n = 7$) (control)	14.5 ± 1.3	6.8 ± 0.7	+ 7.7 ± 1.6	7.7 ± 0.7	9.2 ± 0.5	- 1.5* ± 0.7	- 1.6* ± 0.7	7.6 ± 0.7
Theophylline	15.2 ± 1.7	8.6 ± 0.8	+ 6.6 ± 1.8	7.9 ± 0.9	12.0 ± 1.0	- 4.1 ± 0.7	+ 1.1* ± 1.1	11.8 ± 1.0
<i>P</i>	NS	< 0.01	NS	NS	< 0.01	< 0.01	< 0.02	< 0.001

1.5 $\mu\text{Ci } ^{22}\text{Na}$ and 2.5 $\mu\text{Ci } ^{36}\text{Cl}$ were added to the mucosal solution of one of a pair of tissues and the serosal solution of the other of the pair and, after 30 min equilibration, mucosal and serosal reservoirs were sampled at 20-min intervals. Tissues with electrical resistance differing by less than 25% only were paired to obtain net flux results and electrical values were averaged to yield a single value for each pair. Unidirectional and net fluxes were calculated as previously described⁶. Results are shown for theophylline-treated tissues and paired control tissues in which tissue resistance remained stable throughout the flux experiments. *n*, No. of tissue pairs; + indicates net absorption; -, net secretion. *R*, calculated tissue resistance; Jms, mucosal to serosal flux; Jsm, serosal to mucosal flux; Jnet, net flux. Values are means $\pm$ s.e.m. NS, Not significant.

*Not significantly different from zero.

transport in the presence and absence of glucose (Table 1). In glucose-free buffer theophylline converted a net ileal absorption of sodium to a significant net secretion and chloride secretion was also induced. These changes were due to an increase in unidirectional flux from serosa to mucosa and a reduction in the opposite flux. The results are compatible with both an inhibition of a neutral sodium chloride influx and stimulation of a sodium chloride secretion. But, the decreased epithelial conductance could conceivably account for the reduced Na-Cl influx without invoking any other inhibitory mechanism. However, the increased flux towards the mucosa occurred despite the decreased conductance and the evidence for this secretory process is thus more certain.

Somewhat different results were obtained when glucose was present (Table 1). Here net chloride absorption was changed to secretion but net sodium transport was not significantly reduced. Thus it is possible that theophylline stimulated an uncoupled Cl secretion when glucose was present. The rise in s.c.c. induced by theophylline with glucose ($150 \mu\text{A cm}^{-2}$) was greater than without it ($100 \mu\text{A cm}^{-2}$) in these unpaired studies, possibly due to the separation of anion and cation transport and supporting the possibility of a rheogenic Cl secretion. Furthermore, Sheerin and Field¹⁵ observed that the pH of solutions bathing rabbit ileum influenced the sodium but not the chloride transport response to theophylline. The dependence of sodium secretion on pH suggests that it is not closely coupled with chloride secretion. An uncoupled chloride secretion in response to acetylcholine has also been demonstrated in human intestinal mucosa¹⁶.

But, the evidence for an uncoupled Cl secretion depends critically on the apparent failure of theophylline to influence Na transport in the presence of glucose. It remains possible that the marked movement of sodium from mucosa to serosa induced by glucose masked a relatively small reversal of sodium movement by theophylline.

The increased unidirectional flux of Na-Cl towards the mucosal side due to theophylline plus glucose, might reflect the increased conductance of mucosa. The increase in Cl flux was greater than that in Na (4.5 compared with $3.0 \mu\text{mol cm}^{-2} \text{h}^{-1}$). But, while this discrepancy could be

due to the additional stimulation of a mucosally-directed Cl pump, it could equally be due to a change in the relative conductance of Na and Cl (ref. 6).

Glucose-stimulated bulk fluid transport probably induces appreciable solvent drag forces which increase the mucosa to serosa flux of ions while decreasing that in the opposite direction. This would be more obvious when mucosal conductance is increased. In contrast to this prediction, the Na-Cl flux from mucosa to serosa remained unchanged, suggesting that theophylline might be inhibiting this flux.

In summary, these observations suggest that theophylline exerts several effects. First, it stimulates a sodium and chloride secretory process, seen best in the absence of glucose. This Na-Cl secretion may be a neutral coupled process but a rheogenic chloride secretion may also be revealed in the presence of glucose. In addition, a Na-Cl absorptive flux may be inhibited as deduced from the experiments performed in the presence of glucose.

Finally, it is of interest that human jejunal mucosa also actively secretes chloride in response to theophylline (Table 1) despite the fact that chloride movement here is entirely passive in control conditions, *in vivo*¹⁷ and *in vitro*¹⁸.

C. L. CORBETT
P. E. T. ISAACS
P. C. HAWKER
L. A. TURNBERG

Department of Medicine, Hope Hospital,
University of Manchester School of Medicine,
Salford, UK

Received 25 October 1976; accepted 5 May 1977.

- Field, M. *New Engl. J. Med.* **284**, 1137-1144 (1971).
- Kimberg, D. V. *Gastroenterology* **67**, 1023-1064 (1974).
- Field, M. *Am. J. Physiol.* **221**, 992-997 (1971).
- Schwartz, C. J., Kimberg, D. V. & Sheerin, H. E. *J. Clin. Invest.* **54**, 536-544 (1974).
- Nellans, H. N., Frizzell, R. A. & Schultz, S. G. *Am. J. Physiol.* **226**, 1131-1141 (1974).
- Powell, D. W. *Am. J. Physiol.* **227**, 1436-1443 (1974).
- Evans, D. J., Chen, L. C. & Curlin, G. T. *Nature new Biol.* **236**, 137-138 (1972).
- Corbett, C. L., Isaacs, P. E. T., Riley, A. K. & Turnberg, L. A. *Gut* **18**, 136-140 (1977).
- Frizzell, R. A. & Schultz, S. G. *J. gen. Physiol.* **59**, 318-346 (1972).
- Orloff, J. & Handler, J. *Am. J. Med.* **42**, 757-768 (1967).

- ¹¹ Desjeux, J. F., Tai, Y. H., Powell, D. W. & Curran, P. F. *Biochim. biophys. Acta.* **448**, 352–367 (1976).
- ¹² Nellans, H. N., Frizzell, R. A. & Schultz, S. G. *Am. J. Physiol.* **225**, 467–475 (1973).
- ¹³ Powell, D. W., Farris, R. K. & Carbonetto, S. T. *Am. J. Physiol.* **227**, 1428–1435 (1974).
- ¹⁴ Fromm, D. & Field, M. *Am. J. Physiol.* **229**, 683–688 (1975).
- ¹⁵ Sheerin, H. & Field, M. *Am. J. Physiol.* **228**, 1065–1074 (1975).
- ¹⁶ Isaacs, P. E. T., Corbett, C. L., Riley, A. K., Hawker, P. C. & Turnberg, L. A. *J. clin. Invest.* **58**, 535–542 (1976).
- ¹⁷ Turnberg, L. A., Fordtran, J. S., Carter, N. W. & Rector, F. C. *J. clin. Invest.* **49**, 548–556 (1970).
- ¹⁸ Fromm, D. *Am. J. Physiol.* **224**, 110–116 (1973).

Fast motor units are not preferentially activated in rapid voluntary contractions in man

Most mammalian muscles include muscle fibres of different histochemical types which are organised in separate motor units (a motor unit is a group of muscle fibres innervated by a single motoneurone) with different physiological characteristics¹. When a muscle carries out a slowly graded contraction, the motor units of the slow type are activated before the motor units with a faster contraction time which are also the ones developing more force since they include a larger number of muscle fibres². This orderly recruitment can also be shown in intact man in whom the spike-triggered averaging method further allows the extraction of the mechanical twitch of a single motor unit from the whole muscle force³. In view of the versatility of the human hand movements and considering recent inferential views about variations in the recruitment order of motor units^{4,5}, we had anticipated that the fast motor units could be preferentially recruited in brisk voluntary contractions. This expectation is not borne out by the results presented. On the contrary we found the faster motor units to be recruited after the slow motor units, even in fast contractions.

A total of 54 single motor units were studied in the first interosseus muscle of the hand in five normal adult subjects. Highly selective bipolar electrodes were inserted through the skin and carefully positioned in the muscle belly so as to pick up selectively the action potentials of two to five different motor units at a single site. Each motor unit was clearly identified from background activities, even during strong rapid contractions. The hand was firmly held in a sturdy receptacle. The isometric force for abduction of the index finger was transduced with an Elf-1000-250 Flatline load cell. The mechanical twitch of each motor unit was extracted from the isometric myogram of the steadily contracted muscle by averaging the (FM tape recorded) analogue force signal. The action potential of the chosen motor unit was selected by a window trigger electronic circuit and made to initiate the duty cycle of a Nicolet model 1074 averaging digital computer. Several periods of steady contraction of the muscle were used in order to avoid fatigue effects. Due precautions were taken to automatically exclude mechanical interference from close-range firing of the unit⁶.

The isometric twitches with different contraction kinetics (time-to-peak 65 and 39 ms respectively) shown in Fig. 1; *a*, *b* correspond to two different motor units recorded with the electrode in the same position in the muscle. When the subject contracts his muscle progressively so that the force increases slowly as a ramp function reaching its peak in about 10 s, the slower contracting unit is activated first, and the faster unit is not recruited until several seconds later when the contraction force exceeds 280 g in this example. The sequence is highly reproducible for each recording site⁶.

In brisk contractions the timing of the motor units discharges is difficult to relate with precise levels of the fast-rising muscle force. But a consistent force threshold

of any chosen motor unit can be estimated by requesting the subject to produce a large series of very brief ballistic contractions, each achieving different peak forces from about 10 to 2,000 g. A given unit never discharges for brisk contractions below a certain peak force, while it always fires for contractions with peak force above that value. The method thus facilitates measurement of a true ballistic force threshold which is consistent in repeated trials⁷. The sample records of Fig. 1 *d–g* show that the smaller unit failed to discharge for the weakest contraction (*d*), but was activated in all others, while the larger unit only started firing when the ballistic peak force exceeded 80 g (*g*).

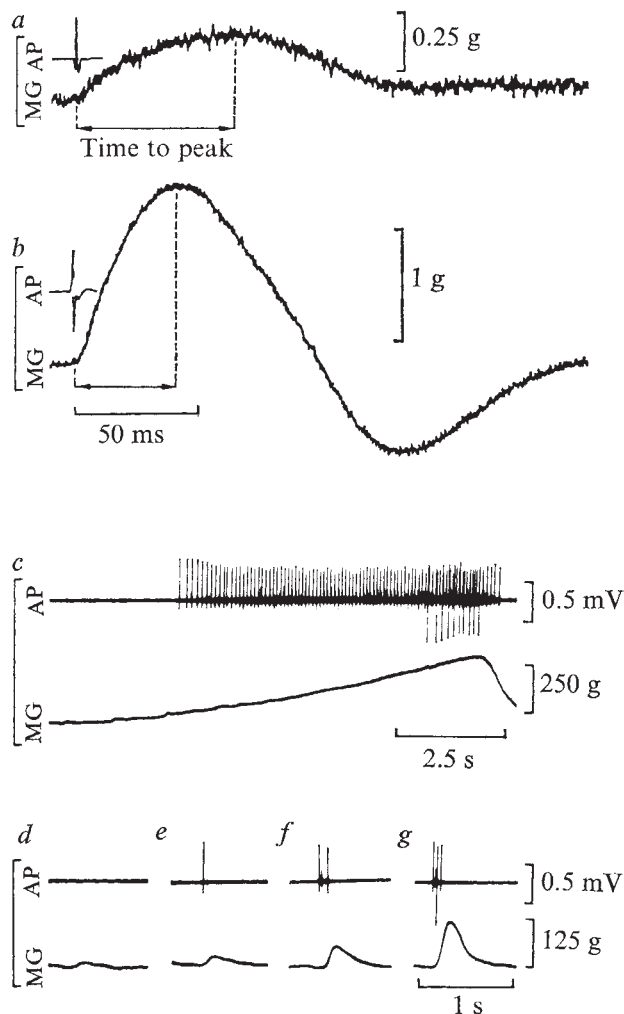


Fig. 1 Recruitment order and force threshold of two motor units in the first interosseus muscle. Normal subject, 28 yr old. The selective recording electrode allowed the action potentials of these two units to be clearly isolated from background activities. The isometric twitch of each unit was extracted by spike-triggered averaging of 512 discharge samples and presents a contraction time (time-to-peak) of 65 ms (*a*) and 39 ms (*b*) respectively. The peak forces are 0.3 g (*a*) and 1.5 g (*b*). The corresponding averaged action potentials (AP) are shown on the same time scale above the myogram (MG). *c*, Voluntary contraction of the interosseus muscle as the subject is tracking a slowly rising force ramp which achieves 300 g in about 10 s. The slower motor unit (illustrated in *a*) starts discharging when the ramp force reaches 20 g while the other motor unit which can easily be identified by its action potential waveform presenting a larger positive-going (downward) component (see *b*, 'AP') only starts discharging later, when the ramp contraction reaches 280 g. *d–g*, ballistic voluntary contractions recorded during the same experiment. The isometric peak forces are 10 g (*d*), 20 g (*e*), 50 g (*f*) and 110 g (*g*) respectively. The contraction in (*e*) just exceeds the ballistic force threshold of the slower motor unit studied. The ballistic contraction in (*g*) recruits the faster motor unit.

For each of the single motor units studied, we obtained complete data for the twitch contraction time, and for the force thresholds respectively in graded ramp and ballistic contractions. Confirming previous results⁸, we found that all the units with a higher ramp threshold were fast contracting. The units with a low ramp threshold were either fast or slow contracting but a significant relation was observed between the two features ($r_{xy} = -0.62$) (Fig. 2a). For the brisk contractions, we found the relation between ballistic force threshold and the twitch contraction time to be rather similar and also quite consistent ($r_{xy} = -0.54$) (Fig. 2b).

The pooled data of Fig. 2 do not allow us to exclude any possible change in recruitment order in the two types of voluntary contractions. This has been achieved by considering pairs of motor units recorded during the same trials from a given position of the recording electrode in

the muscle. Since the specific question considered was whether any reversal of recruitment occurred in brisk contractions, the motor unit pairs considered were required to present significantly different twitch kinetics which we arbitrarily chose to define as a difference of at least 8 ms (range 8–56 ms; mean 23 ms) between their respective contraction times. Twenty-two pairs were retained which satisfied the further criterion that, for any pair, the slower unit had been recruited before the faster unit of the pair in the slow ramp contractions. This was obviously required to answer the question whether any preferential activation of the faster unit occurred in ballistic contraction. However, we did not observe a single instance in which the faster unit of the pair was recruited before the slower unit when the subject produced a brisk ballistic movement instead of a slow tracking ramp.

The wide repertoire of voluntary movements in man and the capacity for brisk and accurate finger displacements is related, amongst other things, to the richness of corticomotoneuronal (pyramidal) connections^{9,10}. When man contracts a muscle so as to track a slowly rising ramp force, the recruitment order of the single motor units is consistent and stereotyped^{6,8} (Fig. 2a). Such movements, even though they are carried out through pyramidal commands on the segmental α motoneurons, involve a co-activation of the γ motoneurons which contract the spindle muscle fibres and thereby elicit an important excitatory drive through the γ loop on to the same α motoneurons¹¹. Spindle afferents feedback excitation was shown in animals to recruit small α motoneurons before larger ones, according to Henneman's size principle^{2,12}. It is thus not surprising that ramp voluntary contractions in man should also obey the size principle. By contrast, when man executes brisk contractions as fast as possible, the pyramidal commands to the α motoneurons must be pre-programmed so as to produce the required ballistic force, and there is no time for any corrective feedback action from spindle or other afferents¹³. In such conditions, the synaptic input to these α motoneurons is different from that in ramp contractions, and we expected to find more flexibility in the recruitment order, if only for allowing fast contracting motor units to be preferentially activated in brisk movements, but our present data definitively exclude such an hypothesis. Both slow and fast motor units containing fibres of either histochemical types respectively occur in the human interosseus muscle¹⁴. The muscle fibre typing is known to be related to the motoneurone properties, including cell size¹. Our findings suggest that the significance of the histochemical and functional types of motor units (slow and fast) should be related, not to any selective usage in either slow or fast voluntary movements in man, but rather to the more frequent activation of the smaller motoneurons according to the size principle¹⁵.

This work was supported by the FNRS and by the FRSM (Belgium).

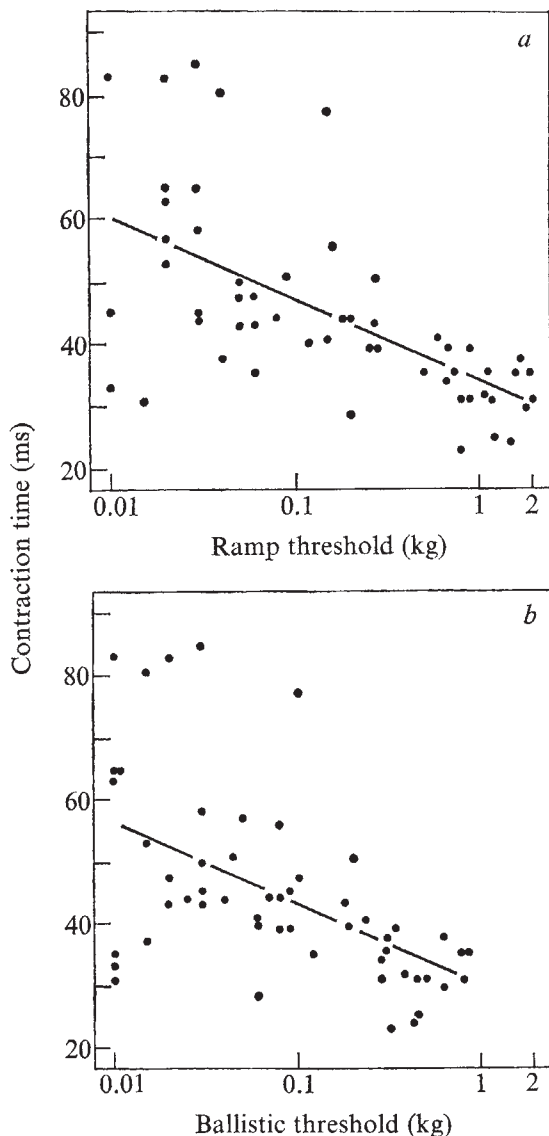
JOHN E. DESMEDT
E. GODAUX

Brain Research Unit,
University of Brussels,
115, boulevard de Waterloo,
1000-Brussels, Belgium

Received 5 April; accepted 3 May 1977.

- ¹ Kugelberg, E. & Edstrom, L. *J. Neurol. Neurosurg. Psychiat.* **31**, 415–423 (1968).
- ² Henneman, E., Somjen, G. & Carpenter, D. O. *J. Neurophysiol.* **28**, 560–580 (1965).
- ³ Milner-Brown, H. S., Stein, R. B. & Yemm, R. *J. Physiol., Lond.* **228**, 285–306 (1973).
- ⁴ Basmajian, J. V. *Science* **141**, 440–441 (1963).
- ⁵ Grimby, L. & Hannerz, J. *J. Neurol. Neurosurg. Psychiat.* **31**, 565–573 (1968).
- ⁶ Tanji, J. & Kato, M. *Expl Neurol.* **40**, 771–783 (1973).
- ⁷ Desmedt, J. E. & Godaux, E. *J. Physiol., Lond.* **264**, 673–694 (1977).

Fig. 2 Relation between the contraction time of the twitch of 54 different single motor units recorded in the first interosseus muscle (ordinate), and the force threshold of each unit (abscissa, logarithmic scale) estimated either with a slow tracking ramp contraction (a), or with brisk ballistic contractions (b). The calculated regression lines fit the functions: $y(\text{ms}) = 34 - 13 \log x(\text{kg})$ (a) and $y(\text{ms}) = 30 - 13.4 \log x(\text{kg})$ (b) respectively. The graph also shows that the absolute for thresholds are smaller in ballistic than in ramp contractions.



- ⁸ Milner-Brown, H. S., Stein, R. B. & Yemm, R. J. *Physiol., Lond.* **230**, 359–370 (1973).
⁹ Kuypers, H. G. J. M. in *New Developments in Electromyography and Clinical Neurophysiology* 3 (ed. Desmedt, J. E.) 38–68 (Karger, Basel, 1973).
¹⁰ Phillips, C. G. *Proc. R. Soc. B* **173**, 141–174 (1968).
¹¹ Vallbo, A. B. J. *Physiol., Lond.* **218**, 405–431 (1971).
¹² Henneman, E. in *The Neurosciences, 3rd Study Program* (eds Schmitt F. O. & Worden, F. G.) 281–291 (MIT Press, Cambridge and London, 1974).
¹³ Kornhuber, H. H. *Kybernetik* **8**, 157–162 (1971).
¹⁴ Johnson, M. A., Polgar, J., Weightman, D. & Appleton, D. J. *neuro. Sci.* **18**, 111–129 (1973).
¹⁵ Salmons, S. & Streter, F. A. *Nature*, **263**, 30–34 (1976).

TEA binding sites in crayfish axon membranes

VOLTAGE-DEPENDENT ion channels are the basis of nerve impulse propagation along the axon. The mechanism of gating and ionic selectivity of these channels remains obscure as long as the membrane molecules participating in these processes are unknown. Some biochemical properties of a tetrodotoxin binding component of the sodium channel have been determined¹, but for the potassium channel not even the number of channels per unit axon surface is known. From noise measurements it has been estimated that the squid giant axon contains about 60 K⁺ channels μm^{-2} (ref. 2), but this figure remains to be confirmed by other methods. Here I report experiments showing that specific and covalent labelling of tetraethylammonium (TEA) binding sites with a radioactive photoaffinity label can be achieved. 4-Azido-2-nitrobenzyltriethylammonium fluoroborate has been shown to block selectively, and after irradiation irreversibly, potassium conductance in the node of Ranvier of frog sciatic nerve³. I present here evidence that in crayfish unmyelinated nerve fibres, the label used reacts with a hydrophobic area at the mouth of the potassium channel postulated by Armstrong⁴.

Walking nerve from *Astacus leptodactylus* was cut into small pieces and 50 mg of tissue was homogenised in 0.2 ml Ringer's solution with a glass Micro-Homogeniser (Micro-Metric Instrument Company, USA). The homogenate was washed three times by centrifugation and resuspension to remove the soluble proteins. The washed membranes were incubated with the photoaffinity label (³H activity 100 mCi mmol⁻¹, synthesised by H. Kiefer, Basle) at final concentration as indicated in the legends to the Figures. After 10 min pre-incubation in the dark, irradiation was performed with an ultraviolet lamp (Sterisol, Quarzlampen GmbH, Hanau, Germany) mounted 5 cm above the sample. The irradiated sample was washed three times by centrifugation and resuspension to remove non-covalently bound photolabel. The labelled membranes were extracted overnight with 1% Triton X-100 in Ringer's solution containing 0.1 mM phenylmethylsulphonylfluoride and 0.02% sodium azide. After 30 min centrifugation at 40,000g the Triton extract was submitted to SDS-polyacrylamide gel electrophoresis⁵.

Figure 1a shows that apart from radioactive material not entering the 10% polyacrylamide gel, there is only one radioactive component migrating slightly slower than the tracking dye. None of the protein bands in the gel contains significant amounts of label. The residual membrane proteins which could not be extracted with Triton (Fig. 1b) were not labelled either. Figure 1c shows that analysis of a labelled membrane which does not contain voltage-dependent potassium channels, namely the postsynaptic membrane from *Torpedo californica* electric tissue. Here the radioactive band of Fig. 1a is missing; instead all the protein bands which have been shown to be part of the acetylcholine receptor complex are labelled. (This is in agreement with the affinity of the acetylcholine receptor for photolabels containing quaternary ammonium groups⁶) The three experiments of Fig. 1 indicate that the photolabel reacted selectively with a component typical for the excitable membrane of the crayfish nerve, presumably a

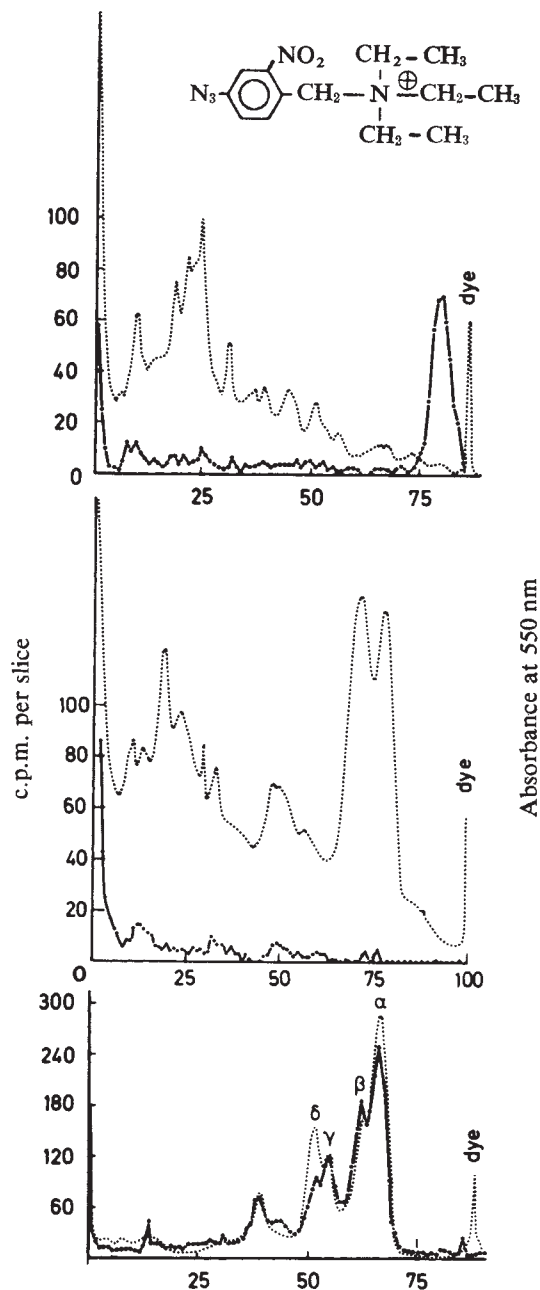


Fig. 1 SDS-polyacrylamide gel electrophoresis of crayfish nerve membranes labelled with the photoaffinity label 4-azido-2-nitrobenzyltriethylammonium fluoroborate. *a*, Absorbance (—) and radio-activity per mm gel slice of a 10% polyacrylamide gel. Walking nerve membranes of *Astacus leptodactylus* were labelled by 2-min irradiation at room temperature of an incubation mixture containing about 1 mg ml⁻¹ membrane protein and 0.5 mM tritiated photolabel in Ringer's solution. Electrophoresis (from left to right) was performed with a Triton X-100 extract of this membrane preparation containing 5 μg protein, at 8 mA per gel. The gels were stained with Coomassie blue. The main radioactive component migrated slightly slower than the tracking dye, the bulk of the lipids (visible as a white band) slightly faster. *b*, Absorbance and radioactivity of a gel of the membrane after Triton extraction. After centrifugation of the axon suspension in 1% Triton X-100 the Triton-insoluble pellet was dissolved in 1% SDS containing 10⁻³ M dithiothreitol. Electrophoresis as in *a*, with 5 μg protein on the gel. *c*, Control with a membrane not containing voltage-dependent potassium channels. Postsynaptic membrane fractions rich in acetylcholine receptor prepared from *Torpedo californica* electric tissue⁷ at a concentration of 0.2 mg ml⁻¹ in Ringer's solution were incubated with 0.5 mM photolabel and irradiated for 2 min. Electrophoresis with 10 μg of the labelled protein was the same as in *a* and *b*. Note that the main radioactive band of the upper gel is missing. Instead, the main proteins of the receptor membrane α – δ reacted with the label although not equally: the δ component incorporated only little radioactivity (as observed before⁸) again confirming the selectivity of the photoaffinity label.

tetraethylammonium (TEA) binding component of the potassium channel.

As further proof of this assumption two criteria of the specificity of a label for a given binding site were investigated: interaction with specific drugs and saturability. Figure 2 shows the concentration dependence of the ligand binding. Saturation was accomplished at an extrapolated incorporation of 12.5 pmol photolabel per μg protein extractable by Triton X-100 and from this result an attempt was made to estimate the density of binding sites in the axon membrane. From electron microscope pictures of a cross section of the nerve (performed by Ms Cahill in Professor Florey's laboratory) we estimated the axon surface area of 1 cm of the nerve. This amount of nerve incorporated 312 pmol of the label or 59×10^{-11} pmol μm^{-2} ; which represents 354 binding sites μm^{-2} . Size and number of fibres varies by orders of magnitude over the cross

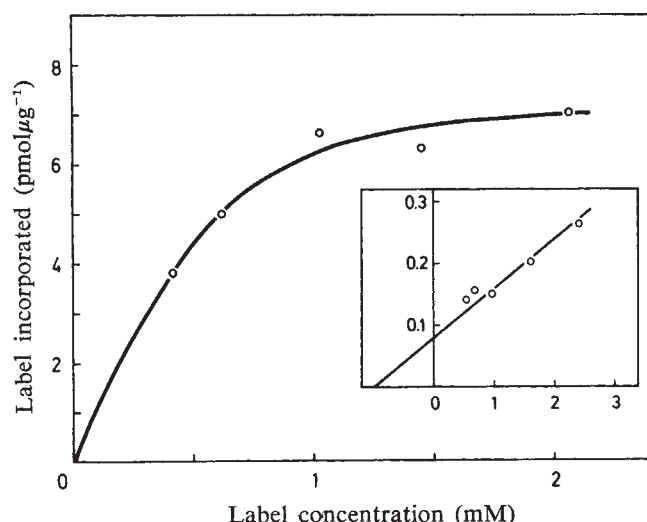


Fig. 2 Concentration dependence of the incorporation of photolabel into crayfish nerve membranes. The ordinate refers to the main radioactive band in the SDS-polyacrylamide gel of membranes prepared, labelled and extracted with Triton X-100 as described. The inset shows the reciprocal plot of the data of the main figure. Extrapolation suggests saturation at 12.5 pmol label per μg protein in the Triton extract. Standardisation with the amount of extractable protein is not intended to suggest that the labelled molecule is a protein. The radioactive band in the gel in fact probably represents a lipid associated with proteins *in situ*.

section of the nerve and so the results from several fairly representative areas were averaged for the calculation. Taking parts of the nerve with small fibres, that is, with large axon surface area, the number of binding sites is about $220 \mu\text{m}^{-2}$. This still seems to be high but since it is preliminary, analogous experiments with squid giant axons are under way.

TEA-C9, the strong inactivator of potassium conductance investigated by Armstrong⁴, protects the membrane against incorporation of photolabel (Table 1). A larger protective effect cannot be expected because during 1 min irradiation the reversible ligand TEA-C9 competes with the covalently reacting photolabel. Treatment of the Triton X-100 extract before the SDS electrophoresis with phospholipase A₂ from hog pancreas (Boehringer, Mannheim, Germany) reduces by about 75% the recovery of radioactivity from the gel; phospholipase D, trypsin and chymotrypsin have no effect. The total radioactivity can be extracted from the membrane with chloroform-methanol 2:1. These results seem to indi-

Table 1 Effect of the TEA analogon TEA-C9 in which one of the ethyl groups is substituted by a nonyl residue

Photoaffinity label (mM)	TEA-C9 (mM)	Inhibition (%)
0.5	10	19
0.25	10	67

The reagent was supplied by E. Rojas, Norwich, England.

cate that the labelled molecule is a phospholipid or a small proteolipid. Armstrong has shown that substitution of one ethyl group of TEA by more hydrophobic residues increases its potency as inactivator of the potassium conductance. This is in agreement with a model of the channel that postulates a hydrophobic area at its mouth. The results presented here may indicate that the photoaffinity label reacted with the lipid rim of the funnel-like entry⁴ to the potassium channel.

The claim that the photolabel selectively reacted with components of the potassium channel is based on three observations: The saturation curve obtained with increasing label concentrations; the competition with the specific potassium conductance inactivator TEA-C9; and the electrophysiological experiments with the frog sciatic nerve³. This last is based on the assumption that the pharmacological properties with respect to TEA and its derivatives are similar in the crayfish axons used here and the frog sciatic nerve investigated previously³. These experiments suggest that photoaffinity labelling might be a valuable tool for the determination of ion channel densities and channel structure in various tissues.

I thank Ms Jutta Birsner and Mr Giampiero Bandini for technical assistance. Ms Grace Chapman introduced me to the preparation of the nervous tissue, and Professor Florey and Dr Walther supported me with helpful discussions. Financial support by the Deutsche Forschungsgemeinschaft, SFB 138, and the Fonds der Chemischen Industrie is acknowledged.

FERDINAND HUCHO

*Fachbereich Biologie der Universität,
775 Konstanz,
Germany*

Received 14 March; accepted 27 April 1977

- 1 Benzer, T. & Raftery, M. A. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3634 (1972).
- 2 Conti, F., DeFelice, L. J. & Wanke, E. *J. Physiol., Lond.* **248**, 45 (1975).
- 3 Hucho, F., Bergman, C., Dubois, J. M., Rojas, E. & Kiefer, H. *Nature* **260**, 802 (1976).
- 4 Armstrong, C. M. *J. gen. Physiol.* **58**, 413 (1971).
- 5 Davies, G. E., & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **66**, 651-656 (1970).
- 6 Hucho, F., Lauer, P., Kiefer, H. & Bandini, G. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2624 (1976).
- 7 Duguid, J. R. & Raftery, M. A. *Biochemistry* **12**, 3593 (1973).

Pentylentetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurones

PENTYLENETETRAZOL (PTZ) and penicillin (PCN) are potent convulsants commonly used to produce focal or generalised epileptiform discharges in the mammalian central nervous system¹. Investigations of the cellular basis for their convulsant effects have been performed in various vertebrate and invertebrate preparations with two basic hypotheses dominating the literature: (1) that the convulsant effect is

produced by pharmacological actions on synaptic transmissions to reduce inhibition²⁻⁸ and/or to enhance excitation⁹, or (2) that the convulsants directly enhance the excitability of neuronal membranes apart from effects on synaptic transmission¹⁰⁻¹⁶. We have studied the mechanism of their convulsant action in a mammalian tissue culture system and report here that both PTZ and PCN selectively antagonise post-synaptic responses to γ -aminobutyric acid (GABA), a putative inhibitory neurotransmitter¹⁷. The results presented here provide direct evidence derived from the mammalian nervous system that the convulsant action of PTZ and PCN may be due to a specific pharmacological action on GABA-mediated inhibitory synaptic transmission.

Mouse spinal cords were dissected from 12-14-d-old mouse embryos and mechanically dissociated; the cells were plated at a density of about 10^6 cells per 35-mm diameter dish and grown in tissue culture for at least 4 weeks before electrophysiological study¹⁸. Mature cultures at this age contain numerous well-differentiated neuronal cell types but in these experiments only the larger diameter (10-50 μ m) multipolar spinal cord (SC) nerve cells were studied. SC cells were penetrated under direct vision on a modified stage of an inverted phase contrast microscope with either 3 M KCl or 4 M K acetate microelectrodes (25-50 M Ω). Use of a conventional bridge circuit allowed simultaneous recording of membrane potential and injected current. All recordings were made in the normal growth medium (90% modified Eagle's medium/10% horse serum); MgCl₂ was added to a final concentration of 10 mM to suppress spontaneous synaptic activity and thus allowed a clearer examination of postsynaptic pharmacology. Glutamate (0.5 M pH 8), GABA (0.5 M pH 3.5), glycine (0.5 M pH 3.5), β -alanine (0.5 M pH 3.5), Na penicillin (0.3 M pH 5.6) (all Sigma) and PTZ (0.3 M pH 6) (K & K) were iontophoresed from high resistance (50-100 M Ω) theta glass micropipettes.

Both PTZ and PCN rapidly and reversibly antagonised the voltage response and conductance change evoked by iontophoresis of GABA without affecting responses to either glycine, β -alanine or glutamate (Fig. 1). The depression of the GABA response by concurrent iontophoresis of convulsant was observed on 25 SC cells with PCN and on

20 SC cells with PTZ, and in both cases the depression was dose dependent. The selectivity of either convulsant's antagonism of GABA responses has been observed on 20 cells examined with both GABA and either glycine, β -alanine or glutamate. Furthermore, PCN applied iontophoretically at currents which produced inhibition of the GABA response did not significantly alter resting membrane potential, input resistance, the inversion potential of the GABA response or the characteristics of the action potential (unpublished observations). Similar observations were made with PTZ except for a slight increase in input resistance noted at high currents. Thus, iontophoretically applied, both PCN and PTZ selectively depress GABA-mediated post synaptic responses on mammalian spinal neurons at doses which have no effect on other membrane properties.

Analysis of GABA dose-response curves obtained during control conditions and during coincident iontophoresis of PTZ or PCN suggests that the convulsants inhibit the GABA response competitively. In addition, the manifest selectivity of both convulsants for GABA also suggests that they both compete for GABA at the GABA receptor and not at the chloride conductance mechanism, as has been suggested previously⁷ for PCN. The finding that PTZ selectively blocks the GABA response in mammalian cultured neurones is in contrast to observations made in the frog spinal cord⁸ where PTZ antagonises the dorsal root potential and the action of GABA, β -alanine and taurine on primary afferent fibres with no ability to antagonise the hyperpolarising responses of these neutral amino acids on motoneurons. The results thus indicate that PTZ and PCN are GABA-specific antagonists in the mammalian central nervous system and support the hypothesis that part of their convulsant action is based on a selective blockade of GABA-mediated inhibition.

ROBERT L. MACDONALD
JEFFERY L. BARKER*

Behavioral Biology Branch,
National Institute of Child Health and Human Development,
NIH, Bethesda, Maryland 20014, and
Department of Neurology,
University of Virginia School of Medicine,
Charlottesville, Virginia 22903

Received 25 February; accepted 3 May 1977.

*Present address: Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Maryland 200140.

- 1 Ajmone Marsan, C. in *Basic Mechanisms of the Epilepsies* (eds Jasper, H. N., Ward, A. A. & Pope, A.) 299 (Little Brown, Boston, 1969).
- 2 Davidoff, R. A. *Brain Res.* 36, 218 (1972).
- 3 Davidoff, R. A. *Brain Res.* 45, 638 (1972).
- 4 Curtis, D. R., Game, C. J. A., Johnston, G. A. R., McCulloch, R. M. & MacLachlan, R. M. *Brain Res.* 43, 242 (1972).
- 5 Clarke, G. & Hill, R. G. *Br. J. Pharmac.* 44m, 435 (1972).
- 6 Wilson, W. A. & Escueta, A. V. *Brain Res.* 72, 168 (1974).
- 7 Hochner, B., Spira, M. E. & Werman, R. *Brain Res.* 107, 85 (1976).
- 8 Nicoll, R. A. & Padjen, A. *Neuropharmacology* 15, 69 (1976).
- 9 Futamachi, K. & Prince, D. A. *Brain Res.* 100, 589 (1975).
- 10 Kao, L. I. & Crill, W. E. *Archs Neurol. (Chi.)* 26, 156 (1972).
- 11 Kao, L. I. & Crill, W. E. *Archs Neurol.* 26, 162 (1972).
- 12 Schwandt, P. C. & Crill, W. E. *Neurosci. Abst.* 2, 266 (1976).
- 13 David, R. J., Wilson, W. A. & Escueta, A. V. *Brain Res.* 67, 549 (1974).
- 14 Williamson, T. L. & Crill, W. E. *Brain Res.* 116, 231 (1976).
- 15 Klee, M. R., Faber, D. S. & Heiss, W. D. *Science* 179, 1133 (1973).
- 16 Partridge, L. D. *Brain Res.* 94, 161 (1975).
- 17 Krnjevic, K. *Physiol. Rev.* 54, 418 (1974).
- 18 Ransom, B. R. & Nelson, P. G. in *Handbook of Psychopharmacology* 2 (eds Iverson, L. L., Iverson, S. D. & Snyder, S. D.) 101 (Plenum, New York, 1975).

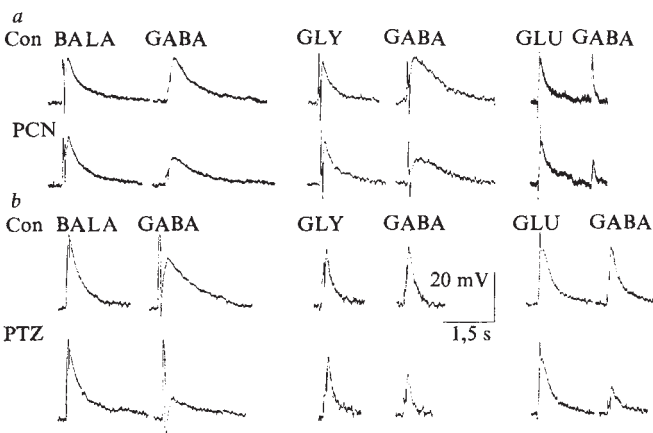


Fig. 1 PCN (A) and PTZ (B) selectively antagonise GABA-mediated postsynaptic responses in cultured mammalian neurones without affecting responses to β -alanine (BALA), glycine (GLY) or glutamate (GLU). All recordings were made using 3 M KCl micropipettes with the cell hyperpolarised to about -90 mV. In these conditions, the inversion potentials for all amino acid responses are more depolarised than -30 mV, and thus all responses shown are depolarising. The amino acids were iontophoresed with 50-ms pulses using micropipettes positioned close to PCN and PTZ pipettes at the neuronal surface. In each case shown, the GABA response was depressed by PCN or PTZ with no change occurring in the other amino acid responses. The 1-s time bar is applicable to all responses except for the GLU and GABA responses in a. Con, control traces.

Reactivation of murine cytomegalovirus by cyclophosphamide

THE high prevalence of cytomegalovirus (CMV) infection in transplant recipients is well known but our understanding of the risk factors involved is incomplete. It is thought that the role of immunosuppression is probably paramount. In a series of rheumatologic patients followed prospectively

Table 1 Reactivation of MCMV in DBA/2 mice by cyclophosphamide

	No. of cyclophosphamide injections			
	0	1	2	3
Salivary gland	0/10*	0/4	11/13	8/8
Kidney	0/10	0/4	3/13	0/8
Lung	0/10	0/4	2/13	0/8
Spleen	0/10	0/4	0/13	0/8
Liver	0/10	0/4	0/13	0/8

MCMV was assayed by plaque formation on monolayers of mouse embryo cell cultures under an overlay containing 0.8% gum tragacanth⁴. Virus titres in the salivary gland ranged from 2.18 to 3.54 log₁₀ PFU per 10 mg of tissue.

*No. of animals with virus in organ/total.

after initiation of cyclophosphamide therapy, a significant proportion (43%) developed CMV infection as evidenced by viruria and serologic response¹. Since most of these infections were seen in initially seropositive patients, we believe that they represent reactivations of latent (herein defined as an infection in which no virus can be detected by standard plaque assays) or inapparent infection. Presumably, reactivation by immunosuppression also occurs in transplant recipients. Another risk factor for CMV infection in renal transplant recipients is the transfer of kidneys from donors seropositive for CMV into seronegative recipients^{2,3}. We are using the mouse cytomegalovirus (MCMV) model for better understanding of the factors involved in latency and reactivation of this group of herpes-like viruses and have shown that allografting will enhance chronic MCMV infection in the recipients⁴. We present here further observations on the mouse model, showing that latent MCMV infection can be reactivated by treatment with cyclophosphamide and that the transfer of latently infected spleen cells can lead to infections in recipients. We also show a role for immunosuppression in the latter situation.

Latent infections were developed by injection of 5–6-week-old DBA/2 inbred male mice (Jackson Labs) with 2×10^5 plaque-forming units (PFU) of MCMV, Smith Strain, intraperitoneally (i.p.). Free virus was no longer detectable in the spleen by 6 weeks after infection. Lungs, liver, kidney and salivary glands were clear by 16 weeks.

Animals that had been infected 20–24 weeks previously were treated with one, two and three doses of cyclophosphamide (150 mg per kg body weight) 5–6 d apart. Their organs were removed, homogenised as 10% suspensions in cell culture medium, clarified by centrifugation and titrated 2 weeks after the last dose (Table 1). Untreated control mice and mice which received only one injection of cyclophosphamide had no detectable free virus in any of the organs tested. On the other hand, 11 out of 13 mice receiving two doses of the drug and all mice treated after three doses reactivated CMV in the salivary gland. The total number of mice in which MCMV was reactivated is

the same as the number of salivary glands positive (Table 1), since MCMV was not found in other organs of mice with MCMV-negative salivary glands. The finding of virus almost exclusively in the salivary gland does not necessarily mean that it is the site of reactivation, as virus in any part of the animal might migrate to the salivary gland, the favoured site of virus replication.

Although investigators could not reactivate herpes simplex virus infections in sensory ganglia of mice using cyclophosphamide⁵, the results presented in Table 1 indicate that cyclophosphamide treatment can reactivate inapparent or latent CMV infections. There is, however, a small but important possibility that the results were produced by amplification of a smoldering but undetected lytic infection. Indeed this possibility is virtually impossible to eliminate.

In an attempt to establish the existence of a transferable, latent CMV infection, spleen cells from DBA/2 mice infected 8–12 weeks previously were used as donors in spleen cell transfer experiments. Recipient DBA/2 and C57BL/10 mice aged 6–10 weeks were treated with a single dose of cyclophosphamide (150 mg per kg) 48 h before injection with 10^8 whole or sonically disrupted spleen cells. Four weeks later, salivary glands from normal and immunosuppressed mice were removed and titrated (Table 2). Transfer of latently infected DBA/2 spleen cells into syngeneic hosts resulted in frequent reactivation in similar proportions of both normal and immunosuppressed animals. Transfer of the same cells into allogeneic C57BL/10 mice, however, resulted in infection in a significantly greater number of treated than untreated recipients ($P < 0.05$)⁶. Free virus was not detected by titration of disrupted donor spleen cells or by injection of 10^8 sonically disrupted cells into immunosuppressed DBA/2 mice. As a control, we performed 'reconstruction' experiments by adding free virus to suspensions of spleen cells before disruption. Recovery of the virus was $>80\%$ using cells derived from either normal or latently-infected animals, suggesting that lytic virus was not inactivated by cell debris or sonication.

These results demonstrate that MCMV may produce latent infections in spleen cells and that this latent infection can be reactivated in other animals by cell transfer. Additionally, in allogeneic recipients, infections due to reactivation of the donated spleen cells were more frequent in cyclophosphamide treated mice than in untreated animals.

The reactivation of latent MCMV by cyclophosphamide provides supportive experimental evidence of the postulated role of immunosuppression in CMV infection of transplant recipients and other immunosuppressed patients^{1,2,7}.

The apparent reactivation of latently-infected spleen cells in non-immune recipients closely resembles the situation seen in kidney transplant recipients. The enhancing effect of cyclophosphamide in allogeneic recipients was probably due to the suppression of rejection of latently-infected transplanted cells, and survival of such cells seems to be the most important factor in determining the frequency of reactivation in this system. A direct effect of cyclo-

Table 2 Effect of transfer of latently-infected DBA/2 spleen cells to normal and immunosuppressed syngeneic and allogeneic recipients

Strain	Recipient Cyclophosphamide†	Inoculum†	Experiment no.				Total
			1	2	3	4	
C57BL/10	—	Live	0/5*	0/5	2/10	—	2/20
C57BL/10	+	Live	3/5	3/10	4/5	—	10/20
DBA/2	—	Live	2/5	2/5	3/5	7/15	14/30
DBA/2	+	Live	4/5	6/10	4/5	5/10	19/30
DBA/2	+	Sonicated	—	—	0/5	0/5	0/10

*No. of animals with virus in salivary gland/total. See Table 1.

†Live, 10^8 spleen cells from latently-infected DBA/2 mice; sonicated, the same number of sonicated cells.

‡+, Cyclophosphamide-treated; —, untreated.

phosphamide on the latently infected donor cells can be ruled out since the drug was received 48 h before transfer of the cells. In syngeneic animals, no rejection would be expected and the lack of effect of cyclophosphamide was a reasonable observation. These experiments add to the accumulating information which supports the concept of immunological involvement of latent CMV infections^{8,9}.

The mouse/MCMV system seems to be an excellent model for human CMV infections in transplant recipients, in that two of the main features, reactivation by immunosuppression and infections caused by transfer of latently-infected tissue into an immunosuppressed recipient can be studied in controlled conditions.

This work was supported by the US NIH (grant no. 5 ROI 11798).

DONALD R. MAYO
JOHN A. ARMSTRONG
MONTA HO

Department of Microbiology,
Graduate School of Public Health,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15261

Received 7 March; accepted 2 May 1977.

- 1 Dowling, J. N., Saslow, A., Armstrong, J. A. & Ho, M. *Yale J. biol. Med.* **49**, 77-82 (1976).
- 2 Ho, M., Suwansirikul, S., Dowling, J., Youngblood, L. & Armstrong, J. *New Engl. J. Med.* **293**, 1109-1112 (1975).
- 3 Nankervis, G. A. *Yale J. biol. Med.* **49**, 27-28 (1976).
- 4 Wu, B. C., Dowling, J. N., Armstrong, J. A. & Ho, M. *Science* **190**, 56-58 (1975).
- 5 Stevens, J. G. & Cook, M. L. in *Perspectives in Virology* **8**, (ed. Pollard, M.) 171-184 (Academic, New York and London, 1973).
- 6 Mainland, D. & Murray, I. M. *Science* **116**, 591-594 (1952).
- 7 Craighead, J. E., Hanshaw, J. B. & Carpenter, C. F. J. *Am. med. Ass.* **201**, 725-728 (1967).
- 8 Olding, L. B., Jensen, F. C. & Oldstone, M. B. A. *J. exp. Med.* **141**, 561-572 (1975).
- 9 Lang, D. J., Cheung, K. S., Schwartz, J. N., Daniels, C. A. & Harwood, S. E. *Yale J. biol. Med.* **49**, 45-58 (1976).

Virus protein p30 in blood predicts development of leukaemia in mice injected with MuLV

ATTEMPTS to monitor by bioassays the fate of murine leukaemia virus (MuLV) inoculated into animals have consistently detected virus in various organs and blood plasma of apparently healthy carrier hosts¹. A correlation of early MuLV titre with spontaneous leukaemia has been demonstrated in mice of the BALB/c × AKR cross². Detection of C-type viral proteins in organs from mice³⁻⁵ has demonstrated a correlation between levels of endogenous viral proteins and the incidence of spontaneous leukaemia. These findings have been confirmed and expanded by the demonstration⁶ that the concentration of the major core protein, p30, of mouse C-type viruses in blood from mice with a high incidence of spontaneous leukaemia (AKR and C58) is a hundred times greater at 2 months of age than in blood of 10 strains with a low incidence of the disease. During a study of the oncogenic potential of an ecotropic MuLV, D2BAL-10, we have found that high concentrations of p30 measured early in life in the blood of animals inoculated at birth with MuLV correlated with subsequent development of leukaemia.

The D2BAL-10 MuLV, isolated from a dimethyl(α)-benzanthracene-induced, transplantable DBA/2 leukaemia, was injected into newborn BALB/c mice. When they were 36 d old p30 was determined by radioimmunoassay⁶ in the blood of individual mice (Fig. 1). In eight of 13 mice injected with virus we found significantly increased levels of blood p30, ranging from 760 to 1,200 ng ml⁻¹, whereas

the remaining five mice had less than 100 ng ml⁻¹. All control animals had blood p30 at concentrations below 50 ng ml⁻¹. Within 247 d all eight animals with increased p30 had either died or been killed in a terminal state of disease. In six of these, the diagnosis, leukaemia, seems to be justified based on macroscopic findings. All had extensive enlargement of the spleen and liver, and in addition one had an enlarged thymus, one had enlarged lymph nodes and one had both. Diagnosis was not possible in the remaining two animals due to cannibalism. One of them, however, had a grossly enlarged spleen. Microscopy confirmed the diagnosis in the four animals which were killed, and from all four it was possible to transplant the disease to syngeneic hosts.

In four of the five virus-injected mice, which initially had low concentrations of blood p30, there was an increase by about 200 d of age. Two of these mice developed leukaemia and were killed at 310 and 380 d, respectively. The remaining three had no signs of leukaemia after 13 months.

One of the control animals with a low p30 concentration in the blood died after 192 d with enlarged lymph nodes and splenomegaly, possibly due to leukaemia. The remaining 12 control animals were alive without signs of disease

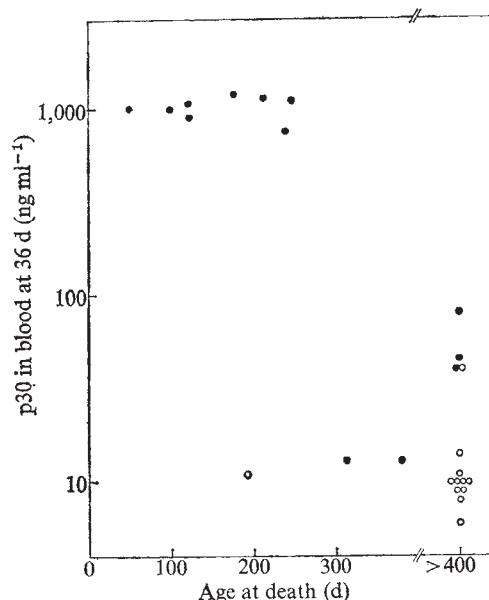


Fig. 1 Newborn BALB/c mice were injected with D2BAL-10 MuLV. Concentration of p30 at 36 d in blood from mice injected with virus (●), and from control mice receiving only buffer + DEAE-dextran (○) is shown in relation to later mortality. A cell-free extract, prepared from D2BAL-10 ascites cells (133 passages), was added to monolayer cultures of SC-1⁷ murine cells. Forty-eight-h collections of MuLV were concentrated 400-fold by producing a pellet of virus from the supernatant medium and resuspending in TEN buffer (20 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 7.6) with p30 at about 0.4 mg ml⁻¹. DEAE-dextran⁸ was added to a concentration of 25 μg ml⁻¹. Each of 13 newborn (less than 24 h old) BALB/cABom-Fib mice received 50 μl of the virus suspension subcutaneously on the back, and 13 newborn BALB/c received 50 μl of TEN buffer with DEAE-dextran (25 μg ml⁻¹). At 36 d a 25-μl sample of blood from each mouse was collected in one tenth volume of 100 mM NaEDTA, pH 6.5. The proteins were solubilised by addition of Triton X-100 to a final concentration of 0.4% followed by freezing and thawing. The radioimmunoassay mixture⁶ contained 0.5 ng of ¹²⁵I-Rauscher-MuLV p30, 12 nl of rabbit anti-FeLV, 1 μl of nonimmune rabbit serum and 10 μl of blood, or a standard dilution of Rauscher-MuLV p30, in a total of 120 μl of TENT/BSA (TEN buffer containing 0.2% Triton X-100, 0.1% NaN₃ and bovine serum albumin (5 mg ml⁻¹)). The antibody-bound radioactivity was precipitated by pig immunoglobulin against rabbit IgG (Dakopatts, Denmark), collected on membrane filters, washed and counted.

after 13 months. They had normal concentrations of blood p30 ($<50 \text{ ng ml}^{-1}$) after 200 d.

These results suggest that the D2BAL-10 MuLV isolated from a long-transplanted, chemically-induced DBA/2 leukaemia is oncogenic in BALB/c mice. It is also obvious that infection of newborn mice with the virus leads to high concentrations of the core protein, p30, in the blood within the first 5 weeks of life in most cases. The increased concentrations of p30 in the blood probably represent replication of the inoculated MuLV in the animals. Finally, there seems to be a significant correlation between early high p30 concentrations in the blood of otherwise healthy animals and the subsequent development of leukaemia. The results agree with the observation that quantification of p30 in the blood identifies among inbred strains of mice, a group which is at high risk of developing leukaemia⁶.

We thank Drs Mette Strand and Thomas August for antiserum, Drs Jack Gruber and George Todaro for help in obtaining Rauscher-MuLV and Dr Walter A. Nelson-Ress for help in obtaining SC-1 cells. We also thank Dr Jes Forchhammer for discussions and Ulla Bockhahn and Arne Jensen for technical assistance. This work was supported by the Danish Medical Research Council. B.A.N. is a research fellow of the Danish Cancer Society.

KAY ULRICH
Bjørn ANDERSON NEXØ

Fibiger-Laboratory,
DK 2100 Copenhagen Ø,
Denmark

Received 3 March; accepted 27 April 1977.

¹ Gross, L. *Acta Haemat.* 29, 1–15 (1963).

² Lilly, F., Duran-Reynals, M. L. & Rowe, W. P. *J. exp. Med.* 141, 882–889 (1975).

³ Aoki, T., Boyse, E. A. & Old, L. J. *J. natn. Cancer Inst.* 41, 103–110 (1968).

⁴ Meier, H., Taylor, B. A., Cherry, M. & Huebner, R. J. *Proc. natn. Acad. Sci. U.S.A.* 70, 1450–1455 (1973).

⁵ Strand, M., Lilly, F. & August, J. T. *Proc. natn. Acad. Sci. U.S.A.* 71, 3682–3683 (1974).

⁶ Nexø, B. A. & Krog, H.-H. *Infect. Immun.* 15, 376–381 (1977).

⁷ Hartley, N. W. & Rowe, W. P. *Virology* 65, 128–134 (1975).

⁸ Ebbesen, P. *Arch. ges. Virusforsch.* 40, 307–310 (1973).

Histone content of germinating pea embryo chromatin decreases as DNA replicates

MODIFICATIONS in the expression of genetic information in eukaryotes are probably controlled by changes in the proteins associated with chromatin¹. Unfortunately, in most of the differentiating systems which have been studied these changes are barely detectable, so that we do not know either which changes occur or when they occur. As a part of a more general study of the molecular developmental biology of seed germination we have investigated changes in chromatin of germinating pea embryo axes. Some particularly interesting features of this biological system make it useful for studying developmental changes in chromatin structure. First, it is quite easy to obtain large numbers of homogeneous seeds. Second, germination constitutes a switch from a dormant, arrested state to a new developmental programme. It is thus possible to study the sequence of molecular events which underlie the transition from a completely inactive genome to an active one. Third, this reactivation of the genome is accompanied by two waves of synchronous mitoses which can be considered as temporal markers. Finally, the germinating embryo constitutes a set of differentiating tissues. To our knowledge, no animal system shares all these features together. The results which are described in the following sections demonstrate that a considerable loss of histone per DNA unit is associated with this reactivation of the

genome during germination and that most of these changes take place as DNA replicates.

Histones from pea embryo chromatin were prepared as described previously; these had been characterised using polyacrylamide gel electrophoresis, electrofocusing and amino acid composition^{2,3}. Briefly, chromatin was prepared from a nuclei-enriched pellet. Most of the contaminating material was removed by several washings and the chromatin was further purified by dissociation in 2 M NaCl followed by gentle reassociation in 0.15 M NaCl. To prevent protease activity, sodium thiosulphate was included in all buffers, and all operations were carried out at 4 °C. That no degradation occurs is indicated by the SDS-polyacrylamide gel electrophoretogram shown in Fig. 1. Molecular weights of the individual histones were in accordance with previously published values^{4,5}.

We first determined the total histone/DNA ratio in

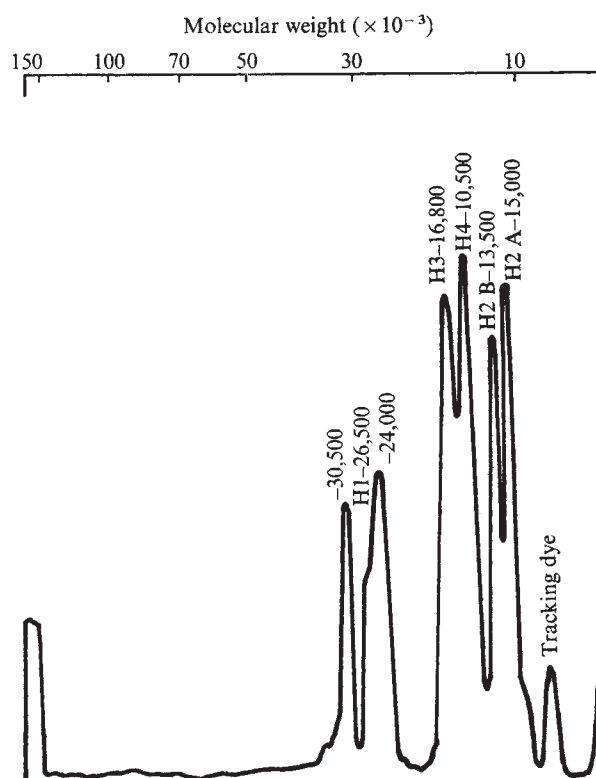


Fig. 1 SDS-polyacrylamide gel electrophoresis pattern of dry pea embryo histones. Chromatin was extracted by homogenisation of embryos in 0.05 M Tris buffer pH 8.0, containing 0.25 M sucrose, 0.001 M MgCl_2 , 0.01 M β -mercaptoethanol and 0.05 M sodium thiosulphate. After filtration of the homogenate through Miracloth, a nuclei-enriched pellet was obtained by centrifugation at 8,000g for 20 min. The pellet was washed three times, twice with the above buffer lacking sucrose and magnesium and finally with 0.01 M Tris buffer, pH 8.0, containing 0.002 M β -mercaptoethanol and 0.05 M sodium thiosulphate. The crude chromatin pellet was then dissociated in 0.01 M Tris buffer, pH 8.0, containing 2 M NaCl and 0.05 M sodium thiosulphate, and gently reassociated by dialysis against the same buffer lacking NaCl, to a final concentration of 0.15 M NaCl. Total histones were extracted by stirring twice with 0.25 N HCl for 2 h and precipitated with eight volumes of acetone at -20°C for 24 h. After centrifugation, the histone pellet was washed successively with acetone and ether and air dried. Histones were analysed by electrophoresis on 12.5% acrylamide gels containing SDS prepared by the method of Laemmli and Favre⁵. Electrophoresis was at 3 mA per gel for 6 h, with bromophenol blue as tracking dye. Gels were stained with Coomassie Brilliant Blue R and scanned at 620 nm with a Chromoscan (Joyce Loebel) apparatus. Molecular weights were estimated by the procedure of Weber and Osborne⁶.

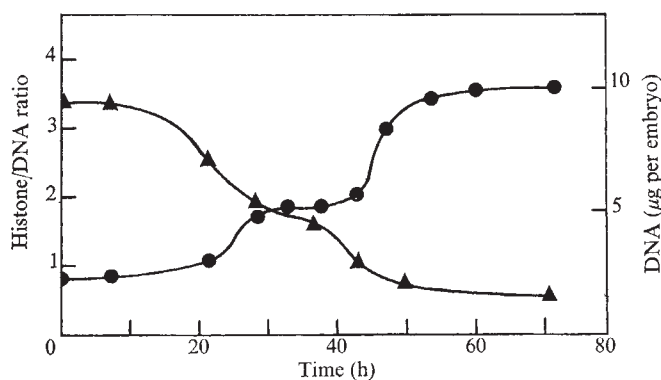


Fig. 2 Changes in DNA amount and in histone/DNA ratio during germination. DNA was extracted by the phenol-chloroform method of Marmur⁷, from embryos germinated for the required period, and assayed spectrophotometrically (●). Chromatin, prepared as described in Fig. 1, was divided into two parts: one for the DNA estimation by the above method, the other for histone extraction (as described in Fig. 1) and estimation by the Lowry method⁹. The histone/DNA ratio of chromatin was calculated (▲).

purified chromatin and examined its variation as a function of germination time. Figure 2 shows the changes in embryo DNA content and in the total histone/DNA ratio. DNA content increases in two steps corresponding to the two waves of mitosis. This has been confirmed by ¹⁴C-thymidine incorporation and cytological observation of numerous mitoses. The histone/DNA ratio curve demonstrates that the histone content of purified chromatin decreases as the germination proceeds. Strikingly, decreases in the ratio are synchronous with DNA synthesis, providing clear evidence that major changes in chromatin take place at the time of DNA replication. Note also that the large excess histone in the dry embryo is most likely accounted for by actual DNA-bound histones than by a storage form, since no free histone can be detected either in the different washings of

the crude chromatin or in the cytoplasm, and since chromatin purified through a sucrose gradient yields the same high histone/DNA ratio (our unpublished results).

We then asked whether each individual histone behaves in the same way or not. Since an accurate quantitation of each individual histone is difficult to obtain by gel electrophoresis, due to differential dye binding, we fractionated the total histone preparation by a selective extraction procedure^{3,8}. Each fraction was checked for purity by gel electrophoresis and assayed by the Lowry method⁹. Using the molecular weights, we calculated the molar percentage of each individual histone and plotted them against time of germination. The data presented in Fig. 3 clearly demonstrate that four of the histones (H2A, H2B, H3 and H4) remain in equimolar proportions although their total amount per DNA unit decreased. This suggests that they remain associated together in a nucleosome-like structure at every stage of germination, including the waves of mitosis. On the other hand, H1 behaved independently. As the germination proceeded, the relative proportion of H1 decreased in steps which, again, showed a remarkable coincidence with DNA doublings.

A direct implication of these observations is that accessibility of DNA in chromatin to endonucleases should increase during the period considered. Chromatin corre-

Fig. 3 Molar percentage of each histone as a function of germination time. Histones were isolated separately by the differential extraction procedure (method 2) of Johns⁸ and assayed by the method of Lowry⁹. In our hands, this yields a fraction corresponding to H2B partially contaminated by H2A. The percentage of contamination was evaluated by electrophoresis and the values corrected. Using the molecular weights as determined in Fig. 1, the molar percentage of each histone was calculated. O, H2A; □, H2B; ▲, H3; ●, H4.

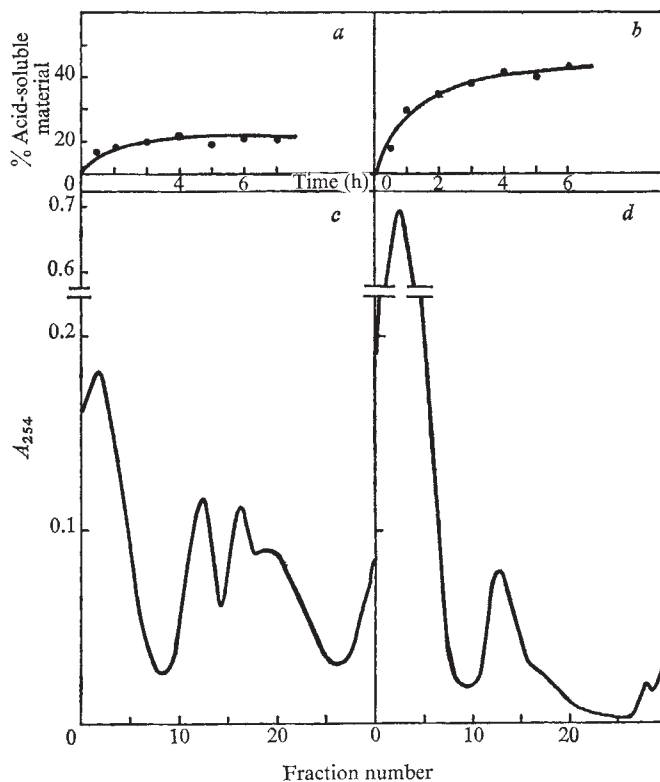
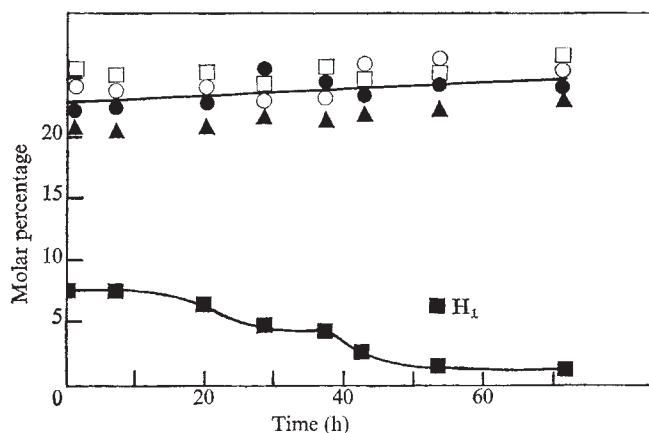


Fig. 4 Digestion of chromatin by DNase II. Mild digestion of chromatin from dry (a and c) and 72-h-old (b and d) embryos was carried out as described previously¹⁰. Each assay contained 5 A_{260} units of chromatin ml^{-1} , and DNase II at a concentration of 6.4 μg DNase per A_{260} unit. Aliquots were taken at various times to test for the production of TCA-soluble material (measured by absorbance of the supernatant at 260 nm after centrifugation at 27,000g and 4 °C for 15 min). The kinetic studies of the hydrolysis (a, b) revealed a rapid initial digestion followed by a slower one. Sucrose gradient analyses (c, d) were carried out during this slower process with chromatin digested for 4 h. An aliquot (1 ml) of each digest was layered on to a 5–20% sucrose gradient. Centrifugation was for 10 h at 40,000 r.p.m. and 4 °C in a Ti SW 41 Beckman Rotor. Gradients were eluted with an ISCO apparatus.

sponding to different stages of germination was hydrolysed with low concentrations of DNase II, and the digests were analysed on sucrose gradients¹⁰. Figure 4 shows two extreme situations, corresponding to dry and 72-h-old embryos. The time courses of the digestions are shown in the top panels. The profiles are similar to those obtained by other workers^{10,11}. The slowest sedimenting particle can be considered as the nucleosome monomer since it exhibits the following properties: it contains all histones except H1, a short DNA molecule homogeneous in size, and has a buoyant density in CsCl of 1.48 g cm^{-3} . Digestion of germinated embryo chromatin (Fig. 4d) yields a larger amount of hydrolysed material than dry embryos (Fig. 4c). These data have been extended to the overall period and the amount of hydrolysed material plotted against germination time (Fig. 5). Obviously the accessibility of DNA increases during germination and the changes correlate with a reduction in the histone/DNA ratio.

Our results provide original information on changes in chromatin structure in the course of a developmental process. They demonstrate that less and less histone is bound to DNA as the genome is re-activated and transcribed. Particularly relevant to the relaxation of the genome was the progressive disappearance of histone H1, the latter being assumed to help in condensing chromatin¹². Note that the level of H1 increased dramatically during seed maturation and thus probably contributes largely to the functional inactivation of the seed genome¹³.

Our results are also in accordance with the observation that H1 is absent or very much reduced in actively transcribed chromatin from animal cells¹⁰. The other four histones remain in equimolar amount and the nucleosome structure is conserved during the overall germination period. As a consequence, the decrease in the histone/DNA ratio and the increase in the DNA accessibility in chromatin can be best interpreted as a decrease in the nucleosome number along the DNA fibre. These changes are correlated

with active transcription of numerous genes¹⁴ and accumulation of non-histone chromosomal proteins¹⁵. This suggests that although nucleosomes are clearly associated with actively transcribed genes¹⁶⁻¹⁹, nucleosome density along the DNA fibre might be a crucial parameter in controlling gene expression. Such a dramatic change can probably be demonstrated only when cell division is required for differentiation and when a large number of genes have to be switched on or off. For example, recent data²⁰ indicate that the differentiation of the avian red blood cell is accompanied by a progressive packaging of more and more histones along the DNA until it is almost completely inactivated. We describe here the reverse situation, in that a repressed genome is reactivated.

In addition, we show that most of the changes involving histones take place at the time of DNA synthesis. This observation has been possible only because most of the cells of the embryo axis replicate their DNA twice with relatively good synchronisation. Note that our results bring direct evidence that when cells divide in a developing system the DNA-bound stock of histone and the chromatin structure and composition are not necessarily identical in mother and daughter cells. This probably constitutes part of the mechanism which allows new sets of genes to be switched on, being a key element in controlling development and cell differentiation.

FRANCOISE GRELLET

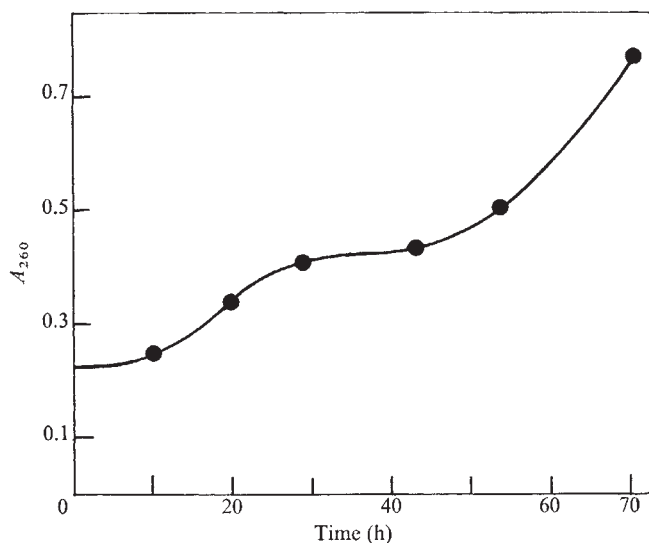
MICHEL DELSENY

YVES GUITTON

Laboratoire de Physiologie Végétale,
Centre Universitaire de Perpignan,
F-66025 Perpignan-Cedex, France

Received 31 January; accepted 27 April 1977.

Fig. 5 Change in DNA accessibility in chromatin during germination. At each stage of germination, the amount of material hydrolysed by DNase II was determined in a series of sucrose gradient experiments as described in Fig. 4c and d. The first seven fractions, corresponding to the absorbing material at the top of the gradient, were pooled and their A_{260} measured.



- ¹ Elgin, S. C. R. & Weintraub, A. A. *Rev. Biochem.* **44**, 725-774 (1975).
- ² Huang, R. C. C. & Bonner, J. *Proc. natn. Acad. Sci. U.S.A.* **48**, 1216-1222 (1962).
- ³ Grellet, F. & Guitton, Y. *Physiol. Veg.* **14**, 453-465 (1976).
- ⁴ Fambrough, D. M. & Bonner, J. *J. biol. Chem.* **243**, 4434-4439 (1968).
- ⁵ Laemmli, U. K. & Favre, M. *J. molec. Biol.* **80**, 575-599 (1973).
- ⁶ Weber, K. & Osborne, M. *J. biol. Chem.* **244**, 4406-4412 (1969).
- ⁷ Marmur, J. *J. molec. Biol.* **3**, 208-218 (1961).
- ⁸ Johns, E. W. *Biochem. J.* **92**, 55-59 (1964).
- ⁹ Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).
- ¹⁰ Oosterhof, D. K., Hozier, J. C. & Hill, R. L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 6633-6637 (1975).
- ¹¹ Altenburger, W., Horz, W. & Zachau, H. G. *Nature* **264**, 517-522 (1976).
- ¹² Barrett, T. *Nature* **260**, 576-578 (1976).
- ¹³ Fambrough, D. M., Fujimura, F. & Bonner, J. *Biochemistry* **7**, 575-584 (1968).
- ¹⁴ Mayer, A. M. & Shain, Y. A. *Rev. Pl. Physiol.* **25**, 167-193 (1974).
- ¹⁵ Grellet, F. thesis, Perpignan (1975).
- ¹⁶ Gottesfeld, J., Murphy, R. F. & Bonner, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4404-4408 (1975).
- ¹⁷ Axel, R., Cedar, H. & Felsenfeld, G. *Biochemistry* **14**, 2489-2495 (1975).
- ¹⁸ Reeves, R. & Jones, A. *Nature* **260**, 495-500 (1976).
- ¹⁹ Weintraub, H. & Groudine, M. *Science* **193**, 848-856 (1976).
- ²⁰ Neelin, J. M., Mazen, A. & Champagne, M. *FEBS Lett.* **65**, 309-314 (1976).

Nature Index and Binders

The complete **Index** for 1976 is available, price £2.50. Copies of the 1975 index are still on sale, price £3.00. **Binders** for the journal are also available at £8.00 for four (a year of *Nature* fits into four binders).

Postage is included in the above prices. Orders should be sent, accompanied by remittance to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, England.

matters arising

Circular superhelical DNA

BRADY *et al.*¹ have presented an excellent X-ray diffraction study of circular superhelical DNA and have shown that the native superhelix of circular DNA from PM2 bacteriophage produces two maxima at angles corresponding to Bragg spacings of 337 and 2,100 Å. We feel that the interpretation given by them for these maxima could be improved in several respects:

(1) The 337 Å maximum is due to superhelical formation by two DNA double helices. Brady *et al.*¹ have neglected the double nature of the superhelix so that their derivation of the

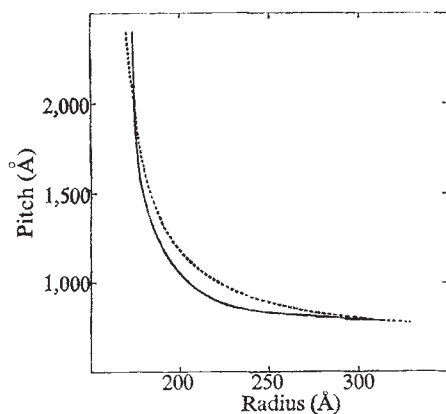


Fig. 1 Helical parameters (p , pitch; r , radius, in Å) for double superhelices which have a scattering maximum at 337 Å. The continuous line has been calculated as described in ref. 3. DNA was simulated by a cylinder of 20 Å diameter. The dashed line is the approximate equation (1) given in the text.

helical parameters is not correct. The first maximum observed will occur in the second layer line. This maximum is approximately related to the helical parameters through the relationship derived directly from the equation for ordered helices²

$$(1/d_{\text{obs}})^2 = (2/p)^2 + (0.487/r)^2 \quad (1)$$

A more accurate relationship can be obtained by numerical calculation from the scattering equation of disordered double helices³. Figure 1 shows that equation (1) describes with sufficient accuracy the relationship between the helical parameters p and r and the position d_{obs} of the first scattering

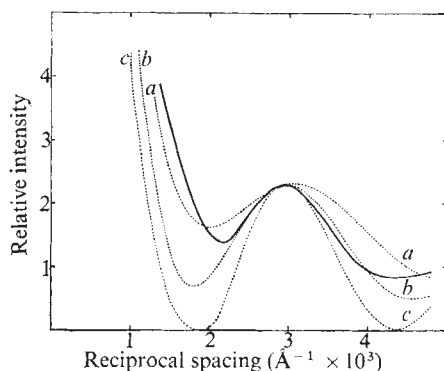


Fig. 2 A comparison of the experimental scattering curve of superhelical PM2 DNA (redrawn from Brady *et al.*¹) and three theoretical curves calculated by the method of Puigjaner and Subirana³: a , $p = 3,300$ Å, $r = 165$ Å; b , $p = 1,440$ Å, $r = 180$ Å; c , $p = 1,020$ Å, $r = 204$ Å. A very shallow maximum is found for large values of the pitch (curve a), since in this case the double superhelix approaches the behaviour of two parallel DNA molecules placed at a mutual distance of about 337 Å. On the other hand, for low values of the pitch ($p < 800$ Å, not shown) the double superhelix approaches a hollow cylinder and new maxima appear, which obscure the maximum due to the superhelical nature of the molecule. Taking into account that the experimental curve has neither been desmeared, nor the background corrected, the dimensions of the superhelix should fall between the values given for the b and c curves.

maximum. In order to determine the actual dimensions of the superhelix, use can be made of the fact that the sharpness of the maximum varies with p and r , as shown in Fig. 2. Unfortunately the experimental limitations¹ do not warrant an exact adjustment of the experimental and theoretical curves, but it can be concluded from such comparison that the radius of the superhelix lies in the range 180–200 Å, with a corresponding pitch in the range 1,400–1,000 Å, dimensions which are about twice those predicted by Brady *et al.*¹

(2) The peak at 2,100 Å has been attributed by Brady *et al.*¹ to a second-order superhelix. To determine its dimensions, Brady *et al.*¹ used an approximate formula which deviates significantly from the calculated values for single superhelices, as shown in Fig. 3. This is due to the fact that scattering in the equatorial plane interferes strongly with the position of the first layer line peak. Furthermore, the 1,23 factor used by

these authors does not seem to be suitable to correct for the random orientation of helices. From the values shown in Fig. 3, and taking into account the shallow shape of the experimental maximum, it can be concluded that the secondary superhelix has a radius of 650 ± 150 Å and a pitch of $2,800 \pm 100$ Å. The pitch can be determined rather accurately, due to the maximum of the theoretical curve shown in Fig. 3. These values indicate that there are three to four second-order turns per molecule.

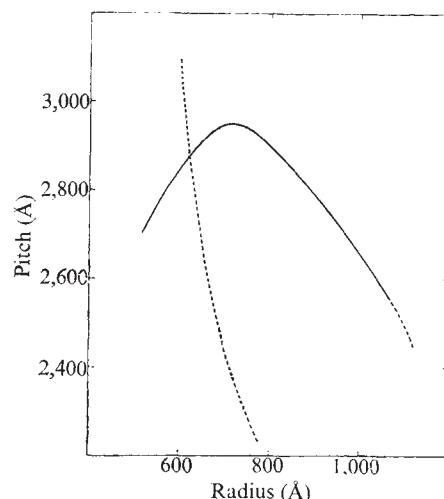


Fig. 3 Helical parameters (p = pitch, r = radius, in Å) for single superhelices which have a scattering maximum at 2,100 Å. The continuous line has been redrawn from the results presented elsewhere (Fig. 3 in ref. 3). The dashed line is the equation (1) used by Brady *et al.*¹. Calculation³ shows that the continuous line cannot be extended towards both ends, since the superhelical maximum is then obscured by diffraction in the equatorial plane of the superhelix. The sharpness of the maximum increases from left to right.

We should also mention, however, that other interpretations for the 2,100-Å peak are possible. It could be due, for example, to formation of a loop, such as a double superhelix, with the distance between the two strands close to 2,100 Å.

JUAN A. SUBIRANA
LUIS C. PUIGJANER

Departamento de Química
Macromolecular del CSIC,
Escuela T.S. de Ingenieros Industriales
Diagonal, 999. Barcelona-14, Spain

- ¹ Brady, G. W., Fein, D. B. & Brumberger, H. *Nature* 264, 231–234 (1976).
- ² Cochran, J., Crick, F. H. C. & Vand, V. *Acta Crystallogr.* 5, 581 (1952).
- ³ Puigjaner, L. C. & Subirana, J. A. *J. appl. Crystallogr.* 7, 169 (1974).

BRADY *et al.*¹ have reported that the X-ray scattering pattern from superhelical PM2 DNA differs from that of its non-superhelical counterpart and interpret their results in terms of unusual tertiary conformational features of the molecule. In so doing they ignore the well documented chemical^{2,3} and enzymological^{4,5} evidence that the secondary structure of superhelical DNA is abnormal and fail to attempt any estimate of the quantitative effect that this may have on their experimental results. In addition, they have ignored the evidence from electron microscopy and light scattering which supports a branched structure for all superhelical DNA molecules with the regions of secondary structure variations situated at the end of the branches^{6,7}. As PM2 DNA possesses between four² and eight³ of such regions the concept of three high-order superhelices distributed over the entire molecule must be equated with there being less than one such turn per branch.

AILS A. M. CAMPBELL

Department of Biochemistry,
University of Glasgow,
Glasgow, UK

- ¹ Brady, G. W., Fein, D. B. & Brumberger, H. *Nature* 264, 231–234 (1976).
- ² Brack, C., Bickle, T. A. & Yuan, R. *J. molec. Biol.* 96, 693–702 (1975).
- ³ Jacob, R. L., Lebowitz, J. & Kleinschmidt, A. K. *J. Virol.* 13, 1176–1185 (1974).
- ⁴ Beard, P., Morrow, J. F. & Berg, P. *J. Virol.* 12, 1303–1313 (1973).
- ⁵ Manjardino, J. & James, A. W. *Nature* 255, 249–252 (1975).
- ⁶ Campbell, A. M. & Eason, R. *FEBS Lett.* 55, 212–215 (1975).
- ⁷ Campbell, A. M. *Biochem. J.* 155, 101–105 (1976).

Down's syndrome in Kerala

KOCHUPILLAI *et al.*¹ reported an abnormally high incidence of Down's syndrome in an area of high background radiation in coastal Kerala. They stated that the radiation exposure of their study group is 1,500–3,000 mrad yr⁻¹. But on the basis of a dosimetric survey² only about 11% of the females in their study received doses in the range of 1,100–2,000 mrad yr⁻¹ and 2.8% received more than 2,000 mrad yr⁻¹. This is due to non-homogeneous distribution of monazite-containing beach sands in the region.

Kochupillai *et al.* observed a frequency of cases of 1 : 1,076 in the study group. Comparison with any other published value is valid if the age structures of the two populations are similar. The infant mortality in this region is about 200 per thousand births³ and is independent of the high background radiation. Furthermore, in most other surveys only 4% of all births have been to females 40 yr old

and above. The data of Kochupillai *et al.* show about 20% of females were in this age group, which incidentally carries the highest risk of bearing children with Down's syndrome. Although in Sweden and Germany 27–29% of the population are in the age group 0–19 yr, in India more than 52% are in this age group³. The greater frequency of Down's syndrome in the study group of Kochupillai *et al.* than in those reported from other countries in ref. 1, could be explained solely in the differences in population structures.

Kochupillai *et al.* say: "Most striking is the higher frequency of cases of Down's syndrome born to mothers aged 30–39 yr (Table 3). This figure of 1 : 81 can be compared with 1 : 880 and 1 : 290 for maternal ages of 30–34 yr and 35–39 yr, respectively, calculated by Penrose and Smith". But the ratio of 1 : 81 relates to the number of cases of Down's syndrome per female in the age group 30–39 yr, and the figures of Penrose and Smith⁷ are for frequencies among all births in these age groups. These are not comparable. Kochupillai *et al.* have demonstrated only that mothers in the age group 30–39 yr run a risk 10 times greater than those in the younger age group, which agrees with other evidence of increasing risk with advancing maternal age. They make no comment as to why this risk is about three times lower in mothers in the age group 40–49 yr. If high background radiation is involved, an even greater risk would be expected in this group than in comparable age groups of the general population. The age-specific risk increases almost exponentially from 1 in 290 at 35 yr to 1 in 46 in mothers aged 45 yr and older⁴.

The incidence of Down's syndrome at birth varies from 1 : 520 to 1 : 873 based on nine surveys¹, with an average of 1 : 663 or 1.507 per 1,000 births for all age groups. Frequencies at birth of around 1 : 800 have been reported for several population groups in India⁵ which experienced normal background radiation. Lejeune⁶ compiled reports which demonstrated no difference between Oriental and Caucasian populations.

Based on values of family size (6.2), the number of females (2,213) in the study population (12,918), an infant mortality rate of 200 per thousand births and a mortality of 60 per 1,000 births in the age group 1–20 yr, a total of 11,500 births is estimated in the study population of Kochupillai *et al.* Using a mean value reported by Penrose and Smith⁷ of 1.507 per 1,000 births, about 17 cases of Down's syndrome would be expected at birth. On the basis of data for the maternal age at birth reported by Verma⁸ and Collmann and Stoller⁴, I have made a more

rigorous calculation, by redistributing the total births estimated into various age groups, on the basis of the number of females in each age group in Table 3 of ref. 1. This gives about 20 cases of Down's syndrome per 11,500 births. (The value should be even higher in this case because 20% of mothers were aged 40–49 yr, unlike any other populations studied.) This figure when corrected for mortality rate⁷ would leave 9 or 10 cases in the population at the time of the study.

Kochupillai *et al.* observed 12 surviving cases of Down's syndrome. The differences between expected and observed are not significant. On the same basis, one would estimate three surviving cases in their control population but none were observed. The differences are not significant. Although it is difficult to explain this, it could have arisen because of the smaller sample size used for the control population as against the study group, and the possible association of a major cardiovascular defect in these cases.

K. SUNDARAM

Bhaba Atomic Research Centre,
Trombay, Bombay 400085, India

- ¹ Kochupillai, N., Verma, I. C., Gewal, M. S. & Ramalingaswami V. *Nature*, 262, 60–61 (1976).
- ² Gopal-Ayengar *et al.*, in *Peaceful Uses of Atomic Energy* 11, 31–51 (International Atomic Energy Agency, Vienna, 1972).
- ³ *Demographic Year Book*, 26th edn, 151–218 (United Nations, New York, 1975).
- ⁴ Hamerton, J. L. *Human Cytogenetics* 11, 196–213 (Academic, New York, London, 1971).
- ⁵ Verma, I. C., & Singh, M. *Lancet* i, 1200 (1975).
- ⁶ Lejeune, J. in *Prog. Med. Genet.* 3 (eds Steinberg, A. G. & Bearn, A. G.) 144–168 (Grune and Stratton, New York, London, 1964).
- ⁷ Penrose, L. S. & Smith, G. F. *Down's Anomaly* (Churchill, London, 1966).
- ⁸ Verma, I. C., Ghai, O. P., Mallick, G. R. & Malhotra, A. K. in *Proc. Symp. Structural and Functional Aspects of Chromosomes*, 330–334 (Department of Atomic Energy, 1975).

KOCHUPILLAI *et al.*¹ reported that the prevalence of mental retardation of genetic origin was four times higher than normal in an area of high background radiation in coastal Kerala. As this background radiation is less than the limit of 5 rem yr⁻¹ recommended for registered radiation workers by the International Commission on Radiological Protection in 1965, this might cause anxiety both to those exposed and to those responsible for their welfare. We therefore wish to point out two reasons why the data are hardly sufficient to imply that the likely risks are so great.

First, 'severe mental retardation' is classified as a 'genetic abnormality'. Other data make it unlikely that more than a small proportion of such cases — of which mongolism (Down's syndrome) is the commonest — are due to any simple excess, deficiency or disturbance of the hereditary material.

Second, an area selected because mongolism was known to be common

was compared with an area in which no case was found in almost 6,000 persons, although one case would be expected in about 700 births.

The only firm data presented show that there were nine cases of mongolism born to 729 women aged 30–39 yr when about two would be expected—and two were born to women 40–49 yr old when about four would be expected. That is, 11 were observed, and, assuming no variation in racial incidence, about six were expected, making possible a very imprecise estimate of perhaps a doubling, perhaps more, but perhaps no increase.

J. H. EDWARDS

Birmingham Maternity Hospital,
Birmingham 15

D. G. HARNDEN

Medical School,
University of Birmingham,
Birmingham 15

¹ Kochupillai, N., Verma, I. C., & Ramalingaswami V. *Nature* 262, 60–61 (1976).

VERMA *et al.* REPLY—The differences in the age composition of the population in India and western countries, which are likely to affect the frequency of Down's syndrome are relatively small (Table 1). Infant and childhood mortality in India is about six times higher than in western countries¹. The mortality of Down's syndrome is expected to be substantially higher than in the general population. We believe therefore that the difference observed in the population frequency of Down's syndrome between the monazite area of Kerala and other countries² is not an artefact due to lack of standardisation for age structure.

We realise that our comparison of the number of cases of Down's syndrome born to women of specified ages with the frequency at birth may not be entirely valid³. Similarly, Sundaram's comparison of 4% of all births to females 40 yr old and above in other surveys with 20% of females in this age group is incorrect. The percentage of women 40–49 yr-old is less in Kerala than in western countries (Table 1).

Sundaram concedes that we demonstrated a ten times higher risk in women 30–39 yr old than in younger

women. This is more than twice the usually reported risk^{4,5}. The risk of Down's syndrome births in the 40–49-yr-old age group is no less than that in the 30–39-yr-old age group if the difference in age-specific fertility (about 56 and 186 per female in the two groups respectively)¹ is remembered.

Calculating the expected number of cases of Down's syndrome in the study population, Sundaram has incorrectly equated the mortality of Down's syndrome with the usual childhood mortality. It is more appropriate to estimate the rate at birth, starting with the observed number of cases: Down's syndrome patients, 12; less than 5 yr, 5; 5–10 yr, 4; more than 10 yr (average age 8.5 yr), 3. Assuming life table survival rates in cases of Down's syndrome in Kerala at 5, 10 and 20 yr as 40, 35 and 30% compared with Collmann and Stoller's figures of 49.4, 46.2 and 38%, respectively⁶ (because of the substantially higher mortality in Kerala), the 12 observed cases would be survivors of 33 liveborn cases of Down's syndrome. Accepting Sundaram's estimate of 11,500 births, the frequency of Down's syndrome would be 2.8 per 1,000 births. This can be compared with a frequency per 1,000 births of 1.5 in the world⁴, 1.2 for an all-India series⁷ (excluding Madras), and 0.8 for Trivandrum, Kerala with normal background radiation (six cases of Down's syndrome among 7,167 births in 1976; personal communication from Saguna Bai).

With respect to the dose of radiation in the monazite belt, we have quoted only published figures^{8–10}. The area surveyed is recognised to have a peak level of radiation. Sundaram's group¹¹ has reported that 80.3% of households in this area recorded radiation exposure greater than 500 mrad yr⁻¹. Almost 27% of households had radiation exposure of 1,000–2,000 mrad yr⁻¹ (Fig. 5 of ref. 11). The dosimetric profile of individuals totally employed in this area is closely similar to the household exposure profile¹¹. With regard to the point raised by Edwards and Harnden, these doses exceed the safe genetic dose recommended by the ICRP for individuals (5 rem yr⁻¹) and for the general population (5 rem per generation but excluding natural background

and medical radiation)¹². The Advisory Committee on Biological Effects of Ionising Radiation sets the limit for the general population at 10 R before the mean age of reproduction of 30 yr (ref. 13).

Edwards and Harnden question our classifying 'severe mental retardation' as a genetic abnormality. This group comprised Noonan syndrome, primary microcephaly, mental retardation associated with craniofacial abnormalities and congenital blindness, and familial cases of mental retardation. No environmental factors were operative in these cases and we think it is reasonable to consider them to be genetic in origin.

The study area was not "selected because mongolism was known to be common". The area was surveyed because it has a high background radiation and a possible relationship with thyroid disease was being investigated¹⁴.

I. C. VERMA

N. KOCHUPILLAI

M. S. GREWAL

K. RAMACHANDRAN

V. RAMALINGASWAMI

Department of Paediatrics,
Medicine, Anatomy, Biostatistics
and Pathology,
All India Institute of Medical Sciences,
New Delhi 110016, India

¹ National Sample Survey Report, No. 186 (Cabinet Secretariat, Government of India, 1971).

² Kochupillai, N., Verma, I. C., Grewal, M. S. & Ramalingaswami, V. *Nature* 262, 60–61 (1976).

³ Penrose, L. S. & Smith, G. F. *Down's Anomaly* (Churchill, London, 1966).

⁴ Hamerton, J. L. in *Human Cytogenetics*, 2 (Academic, London, 1971).

⁵ Verma, I. C., Gupta, A. K. & Devangondi, B. S. *Indian Pediatr.* 12, 1239–1245 (1975).

⁶ Collman, R. D. & Stoller, A. J. *ment. Defic. Res.* 7, 60–68 (1963).

⁷ Verma, I. C. & Singh, M. B. *Lancet*, i, 1200 (1975).

⁸ Gruneberg, H. *et al. Med. Res. Council Spec. Rep. Ser.* 307 (1966).

⁹ Ponnuni Kartha, K. I. *Hlth Phys.* 15, 368–369 (1968).

¹⁰ WHO tech. Rep. Ser. 166, 23 (1959).

¹¹ Gopal Ayengar, A. R. *et al. in Peaceful Uses of Atomic Energy*, 2, 31–51 (International Atomic Energy Agency, Vienna, 1972).

¹² Recommendations of International Commission on Radiological Protection, ICRP Publ. no. 9 (Pergamon, Oxford, 1966).

¹³ Rep. Advisory Committee on Biological Effects of Ionising Radiations. (National Academy of Sciences, Washington DC, 1972).

¹⁴ Kochupillai, N., Thangavelu, M. & Ramalingaswami, V. *Ind. J. med. Res.* 64, 537–544 (1976).

¹⁵ UN Demographic Yearbook (United Nations, New York, 1966).

Table 1 Age structure of population of females

Age group (yr)	Kerala ¹⁴		India* 1961	Australia 1965	UK* 1966	Denmark* 1964
	Study	Control				
1–14	43.39	38.57	41.14	29.06	21.83	23.09
15–19	13.54	12.66	8.12	8.79	7.39	8.71
20–29	13.64	16.49	17.47	13.31	12.61	13.45
30–39	11.53	12.73	12.55	12.45	11.70	12.04
40–49	8.42	9.11	8.97	12.49	12.69	12.88
50 & <	9.48	10.42	11.75	23.90	33.78	29.83

Figures are percentages.

*Figures from ref. 15.

Virus particles in marmoset hepatitis

SERUM from marmosets infected with the GB hepatitis agent has been reported (Almeida *et al.*¹) to contain aggregates of particles, 20–22 nm in diameter, and it was suggested that these particles may be the aetiological agent of GB hepatitis. Dienstag *et al.*² subsequently described the occurrence of 34–36-nm particles in homogenates of livers from marmosets infected with

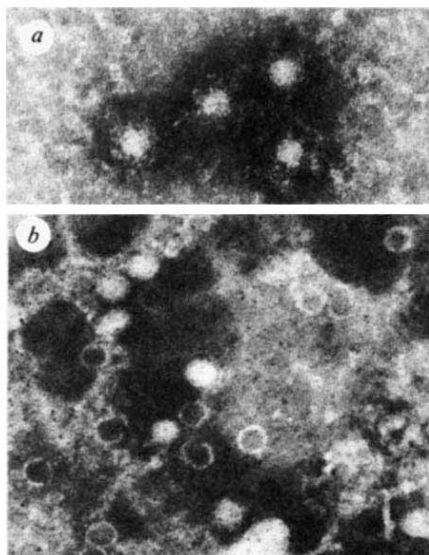


Fig. 1 *a*, 22-nm particles present in marmoset serum. These particles are apparently coated with antibody ($\times 168,540$). *b*, 22–24 nm particles found in marmoset faeces ($\times 168,540$).

the GB agent, but antibody to these particles could not be detected in convalescent serum from the patient (GB) himself. Findings from marmoset transmission studies in this laboratory support the suggestion that the small serum particles described by Almeida may be aetiologically associated with hepatitis in marmosets.

Serum from cotton top marmosets (*S.oedipus oedipomidas*) which had been infected with the Berlin agent which is antigenically related to GB contained small aggregates of round virus-like particles approximately 22 nm in diameter (Fig. 1*a*). These particles seemed to be heavily coated with antibody. Since this serum pool is highly infectious for marmosets presumably free virus particles exist, but electron microscopy is not a sufficiently sensitive technique to be able to detect these.

Similar 22–24-nm round virus-like particles were transiently excreted in the marmoset faeces. Particles were not found in specimens collected on the seventh and twelfth day after inoculation, but a stool specimen collected from one animal on the tenth day contained both full and empty particles (Fig. 1*b*). These particles did not occur in aggregates and there did not seem to be any antibody on them. Levels of serum transaminases were first found to be significantly raised in the serum sample collected on the eleventh day.

The occurrence of 20–24-nm particles in serum of marmosets infected with both the GB and Berlin hepatitis agents, and the transient excretion of similar particles in faeces at the time when levels of serum transaminases were elevated, suggests that these small

virus-like particles may have a significant role in the development of Berlin and GB hepatitis in marmosets.

Marmosets infected with the GB and Berlin agents did not develop antibody to the parvovirus-like antigen found in human sera by Cossart³.

HAZEL APPLETON

Virus Reference Laboratory,
Central Public Health Laboratory,
Colindale Avenue,
London NW9, UK

¹ Almeida, J. D. *et al.* *Nature* 261, 608–609 (1976).

² Dienstag, J. L., Wagner, J. A., Purcell, R. H., London, W. T. & Lorenz, D. E. *Nature* 264, 260–261 (1976).

³ Cossart, Y. E., Field, A. M., Cant, B. & Widdows, D. *Lancet* i, 72–73 (1975).

DIENTSTAG REPLIES.—Appleton has demonstrated 22-nm virus-like particles in serum from *S. oedipus* marmosets infected with the Berlin agent, antigenically related to the GB agent¹. Like those in the report by Almeida

as presumed by Appleton, is highly unlikely. Whether these antibody-coated particles have been sought in non-infectious serum (pre-inoculation, convalescent, and/or normal marmoset serum) is not detailed in Dr Appleton's communication. Even if these antibody-coated particles cannot be detected in normal marmoset serum, as Almeida *et al.*² reported, conclusions about the aetiological role of these particles are unwarranted, for the same reasons we pointed out in our letter⁴.

Regarding the detection of 22–24-nm particles in stool of one infected marmoset on day 10 but not day 7 or 12 after inoculation, the obvious questions are (1) whether pre-inoculation and convalescent stools were also examined, and (2) whether these particles can be aggregated by convalescent but not pre-inoculation serum from Berlin agent-infected marmosets. In our laboratory,

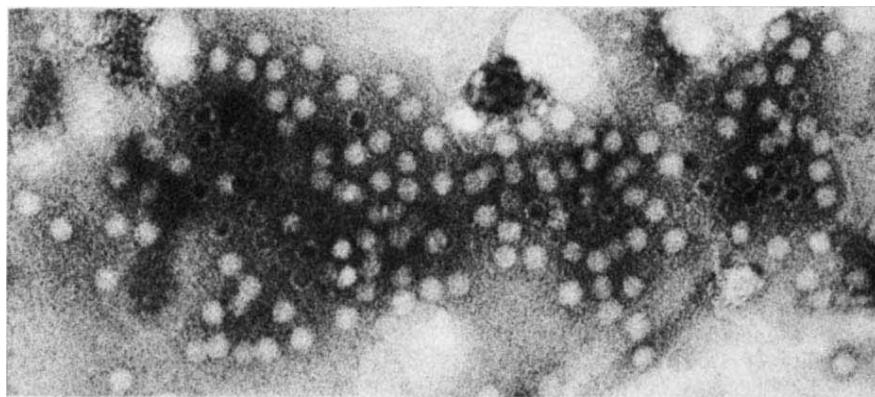


Fig. 1 Electron micrograph of 22-nm particles in the stool of a *S. mystax* marmoset. Such particles appear in normal marmoset stools as well as in stools of marmosets infected with hepatitis A virus and bear no serologic relationship to any known infection. (2% phosphotungstic acid negative stain) ($\times 132,000$).

*et al.*², the particles shown are heavily coated with antibody. Such a dense halo of antibody molecules and the large interparticle distance are characteristic of extreme antibody excess, rather than antigen excess³; therefore, the presence of antibody-free particles,

we have detected morphologically identical 22-nm particles (Fig. 1) in normal marmoset stools, in pre-inoculation, acute illness, and convalescent stools from marmosets infected with hepatitis A virus. These ubiquitous particles bear no serological relationship to any known illness and are easily distinguished from 27-nm hepatitis A antigen particles by immune electron microscopy⁵.

JULES L. DIENSTAG

Gastrointestinal Unit,
Massachusetts General Hospital,
Boston, Massachusetts 02114

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

¹ Appleton, H. J. *Infect. Dis.* 132, 500–505 (1975).

² Almeida, J. D. *et al.* *Nature* 261, 608–609 (1976).

³ Dienstag, J. L., Alling, D. W., Purcell, R. H. *Infect. Immun.* 13, 1209–1213 (1976).

⁴ Dienstag, J. L., Wagner, J., Purcell, R. H., London, W. T. & Lorenz, D. E. *Nature* 264, 260–261 (1976).

⁵ Feinstone, S. M., Kapikian, A. Z. & Purcell, R. H. *Science* 182, 1026–1028 (1973).

reviews

White beard through the looking glass

Richard Dawkins

Behind the Mirror: A Search for a Natural History of Human Knowledge. By Konrad Lorenz. Pp. 261 (Methuen: London, 1977.) £4.90.

A PURELY PERSONAL PREJUDICE leads me to appreciate a book more if I am told, at the outset, what it is the author hopes to teach me. In place of the introductory chapter which might have served this purpose, *Behind the Mirror* begins with 'Epistemological Prolegomena'—long on Kant but, apart from a cryptic last sentence, short on statements of intention. In the final chapter we learn of the author's aim to "attempt to give a survey of man's cognitive mechanisms", something that "no-one else has hitherto ventured to do". "Cognitive mechanisms" are interpreted liberally to include not only higher conscious faculties but also more rudimentary mechanisms of adaptive behaviour which might represent the evolutionary forerunners of consciousness.

The readership at which the book is aimed is also hard to pin down. It is certainly too cerebral for the mass audiences of *King Solomon's Ring* and *On Aggression*, although there are flashes of the humour and the eloquence that characterise those works. On the other hand, although there is a bibliography to match the undoubted erudition of the author, the text lacks the detailed citations which would encourage the professional scholar to follow up points of special interest.

Librarians should file it half way between Philosophy and Ethology, which is another way of saying that I am only half competent to review it. I lack the knowledge (to say nothing of the confidence) to make statements like "The only philosopher ever to voice similar opinions is the Austrian F. Decker". And I cannot professionally evaluate Lorenz's central philosophical point, which seems to me rather important, that "in the interests of objectivity a scientist must understand the physiological and psychological mechanisms by which experiences are conveyed to men... for the same reasons that a biologist must know his microscope...".

The early chapters include some of the arguments given in *Evolution and Modification of Behaviour* (1966). Certainly these points can bear repetition, having been much misunderstood by Lorenz's critics from the school of Hysterical Environmentalism. Animal behaviour is adapted to its environment. This means there is a sense in which facts about the

world are represented in the animal's nervous system or other parts of its physical structure. Just as a key embodies information about the lock which it is manufactured to fit, so even the simplest protozoan contains knowledge about its environment in the sense that it is equipped in advance to survive in that environment.

Those who have ranted against Lorenz's usage of "innate" have sometimes failed to appreciate that he applies the word not to behaviour itself but to the "adaptive information" of behaviour. This is vividly expressed in his treatment of learning: "Unless one believes in supernatural factors... one has to postulate the existence of innate teaching mechanisms in order to explain why the majority of learning processes serve to enhance the organism's fitness for survival. These mechanisms also meet the Kantian definition of the *a priori*: they were there before all learning, and must be there in order for learning to be possible".

In later chapters of *Behind the Mirror* Lorenz comes on to human culture. As always what he has to say is stimulating, but his comparison of biological and cultural evolution is marred by his unfortunate misconception about how evolution works, and therefore about the nature of adaptation. A junior ethologist is bound to be mindful of Lorenz's own emphasis, reiterated in *Behind the Mirror*, on the biological value of reverence for the ancestral wisdom of tribal elders. This is enough to arouse twinges of remorse over the tone of my own recent criticism of Lorenz's unconscious group selectionism. Perhaps it was unfair to pick on *On Aggression* which, after all, was first published in 1963, just before the reaction to Wynne-Edwards brought us back to our neo-Darwinian senses.

But it seems that nothing has changed. In *Behind the Mirror*, animals are still expected to behave for the good of the species, and

there is still no inkling that the issue is the slightest bit controversial. Lorenz sees a "balance between the factors that make for the invariance of the gene pool and those factors that make for its modification..." which "is adjusted to the degree of variability of the environment". Mutation rate is at its "highest in creatures that inhabit highly variable environments". This last statement fits Lorenz's world view like a glove, but it ruffles all my selfish-genetic hackles and I would have liked to have been told where I could look up the evidence.

The idea of a balance between mechanisms for and against change is developed in the analogy of cultural evolution, where it is thought to include genetically programmed tendencies on the part of the young to revere the wisdom of elders—the White Beard Effect—and at other times to rebel against it. Lorenz gives us some fascinating examples of the predominance of blind biology-like evolution rather than rational planning in the development of human culture, and he correctly identifies natural selection as the common factor. But his is a natural selection between large units—species in the biological case, civilisations in the case of culture—rather than, as it should be, natural selection *within* such large units. If you want a cultural analogue of the fundamental unit of biological natural selection, you should seek the analogue not of the gene pool but of something like the gene.

A reviewer is bound to mention the shortcomings he sees in a book, but in this case they are not new and therefore do not diminish his deep respect for the author's achievements, a respect which is far too solid and real to owe its origin to any White Beard Through the Looking Glass. □

Richard Dawkins is Lecturer in Animal Behaviour in the Department of Zoology, University of Oxford, UK.

Language analogue project

Language Learning by a Chimpanzee: The LANA Project. Edited by D. M. Rumbaugh. Pp. 312. (Academic: New York and London, 1977.) \$17.50; £12.40.

LANA is a laboratory chimpanzee, born in October 1970, who has been learning a specially-designed 'language' and engaging in a number of related experiments at the Yerkes Primate Center of

Emory University, Atlanta, Georgia, since she was just over two years old. The 'language' that she is learning is called, appropriately enough, 'Yerkish'; and her own name is identical with the acronymic abbreviation of the formal title of the project; the LANA project is, in full, the Language Analogue project. With the publication of the present volume, which reports the results of the first three and a half years

work, Lana bids fair to becoming as famous as her conspecifics, Washoe and Sarah, trained by the Gardners and the Premacks, respectively. And deservedly so. The LANA project confirms some of the more interesting, not to say exciting, findings of the earlier research; and it makes its own methodological (and to a lesser extent, theoretical) contribution.

The book falls into five parts, consisting in total of sixteen chapters. There is also a very brief foreword by Karl H. Pribram, in which the point is made that "Washoe, Sarah and Lana—and a whole new generation of scientists and apes—are attempting to spell the end of Cartesian dualism". It is also suggested, rather misleadingly to my mind, that "a great deal can be learned about human language from a careful reading of the early chapters". Ernst von Glasersfeld's "Linguistic Communication: Theory and Definition" (chapter 2) does little to advance our understanding of either the structure or the functions of language. Richard K. Davenport's "Cross-Modal Perception: a Basis for Language?" (chapter 3) is no more than suggestive: it is an extremely important finding for comparative psychology that certain non-human primates are capable of cross-modal perception, but the implications of this finding in relation to theories of the nature and origin of language are not drawn in detail.

The most substantial contribution in part I is chapter 1, by Gordon W. Hewes, on the history and current state of speculation about the origin of language. Hewes (who is the only one of the fourteen contributors not to have worked on the LANA project and been responsible for aspects of its design, its day-to-day operation, or the running of some of the experiments) has probably done more than anyone else to resuscitate interest in glottogenic studies and, more particularly, to revitalise, in the light of recent work in several disciplines, the eminently plausible hypothesis that human language originated in gesture, rather than vocal signals. His chapter, as one would expect, is informed and judicious; although it is rather too sketchy in parts and suffers, in the present context, from being geared, as far as references are concerned, to his recent two-volume bibliography. It will no doubt, however, serve the purpose for which it is intended, that of putting the LANA project and other work in "the Chimpanzee Era of glottogenic research" (pp 38–42) in its historical and currently multidisciplinary context.

Lana's training has been more similar to Sarah's than to Washoe's, being based (although less strictly than Sarah's) on the principles of operant

conditioning. Yerkish, too, is like Sarah's plastic-token 'language' in that its grammatical structure and the form and meaning of its 'words' (combinations of up to nine readily discriminable line-figures superimposed on a coloured background) have been fixed in advance by its designers and are rigidly controlled. The syntax is very simple, although otherwise not strikingly different from that of many natural languages in the use that it makes of word order, sentence particles and various predicative relationships. There is provision for co-ordination and embedding. But so far this facility has not been used in the training programme. Nor have other complexities that it is planned eventually to build into the system. It is probably fair to say that Lana's progress with Yerkish is comparable with the progress that Washoe and Sarah made with their 'languages'. It is still an open question, therefore, and to some considerable extent a matter of definition, whether apes have been shown capable of learning a communication system that is rightly des-

cribed as a language. But the research is undeniably of great importance, and the results that have been obtained so far are challenging.

The principal methodological contribution of the LANA project comes from the total automation and computerisation of the process of recording and analysing all the strings of symbols, both by Lana herself and by her 'interlocutors', at all times of the day. As the contributors point out, this has considerable implications for the development of further hypotheses and the analysis of experimental results. The central chapters of the book provide a full account of both the software and the hardware, of the training programme, and of a variety of experiments carried out so far. Much of this has been reported already at conferences and in the specialist journals; but it is convenient to have it brought together in a single volume.

John Lyons

John Lyons is Professor of Linguistics at the University of Sussex, UK.

Pharmacology of the synapse

Chemical Pharmacology of the Synapse. By D. J. Triggle and C. R. Triggle. Pp. viii+654. (Academic: London and New York, 1976.) £20; \$43.75.

THE five chapters in this book cover the structure and function of the synapse (127 pages); quantitative aspects of ligand-receptor interactions (102 pages); structure-activity relationships: chemical constitution and biological activity (197 pages); the molecular basis of neurotransmitter-receptor interactions (163 pages), and characterisation, quantitation and isolation of neurotransmitter receptors (41 pages). It therefore attempts to cover biological and chemical topics which are of fundamental importance in the understanding of the actions of drugs at receptors.

The first chapter should be helpful as an introduction for chemists and biochemists. The second deals with the relation between dose and response in experiments where this is not complicated by metabolism and transport. Some pharmacologists may feel that the treatment is not as rigorous as it might be, but it is a fair account of current ideas. The third chapter reviews structure-activity relationships at eight separate receptors, and although this is the biggest chapter in the book and

contains much useful information, it is rather fragmentary. Each receptor really requires more extensive discussion and the non-chemical reader may find this chapter heavy going. It also needs some underlying idea to hold the eight sections together and an attempt to supply this is made in the fourth chapter. In our present state of knowledge, however, the molecular basis of neurotransmitter-receptor interactions is very uncertain and the chemist may feel disappointed when faced with an account that is inevitably largely electrophysiological. Understanding the basis is, in fact, going to require further information, which should come from studies with isolated receptors, dealt with briefly in the last chapter. The book, therefore, does not supply all the answers, but it gives a reasonable account of the problems and of the work which has been done to try to solve them.

There are some blemishes. The authors ought to make up their minds whether to use (*d*) or (+) for the sign of rotation and the formula for nicotine is twice given incorrectly (p22, p292); only half the absolute configuration is indicated in the formula of (+)-tubocurarine (p23).

Although most readers will probably find something to object to somewhere in the book, the authors have done a useful public service in collecting together so much information, much of it recent. The subject needs a book of this sort, which will make a useful starting point for students who are new to this kind of work, whether they are

chemists, biochemists, pharmacologists or physiologists. All these will find some part of the book where they will feel at home, and this should encourage them to see their contribution in relation to that of others. Although it may be difficult to write a book all of which will appeal to readers with such different backgrounds, the subject is

not divided up in the way that scientists are. This book is important because it attempts to review synaptic events as a whole.

R. B. Barlow

R. B. Barlow is Lecturer in Chemical Pharmacology at the Medical School, University of Bristol, UK.

Recognising variable patterns

Syntactic Pattern Recognition: Applications. Edited by K. S. Fu. Pp.xi+270. (Springer: Berlin and New York, 1977.) DM 85; \$37.40.

THIS collection of ten papers by different authors is concerned with the application of automatic pattern recognition to electrocardiograms, speech, chinese ideograms, mathematical expressions, finger prints, remotely sensed imagery and parts of industrial assemblies. The central technical problem is that machines are required to recognise patterns that are not unique. For instance, countless minutely different waveforms should be recognised as clinically normal electrocardiograms. Similarly, two successive finger prints from one right thumb will generally be slightly different, depending for instance on distortion of the skin by pressure while the print is taken. Such differences often make it unexpectedly difficult to make machines recognise what the brain can easily recognise.

There is at least a tenuous analogy between deciding which patterns are *bona fide* finger prints of one right thumb and deciding which strings of symbols constitute a syntactically admissible sentence in a formal language. In both cases a large or infinite set of strings or patterns has to be defined by means of definitory apparatus that contains relatively little information. Furthermore, a finger print consists for instance of a whorl or tented arch which in turn consists of minutiae in various positions; and similarly a sentence consists of phrases which in turn consist of words. This illustrates the general idea that patterns, as well as sentences, can usefully be subject to a hierarchical structural description as in parsing. Because formal language theory is well understood, various workers have tried since about 1964 to do some good with it in automatic pattern recognition.

Chomskian grammars turn out to be

too abstract, too simple, for immediate application to pattern recognition. First, a phrase structure is concerned only with concatenation of symbols, not with the details of their relative positions and shapes. Second, a simple grammar is one-dimensional, whereas finger prints are not. Both of these problems can be overcome to some extent, and the book under review gives usually *ad hoc* details for the different practical applications.

The first chapter is a concise introduction by Fu, whose textbook *Syntactic Methods in Pattern Recognition* (Academic Press, 1974) makes an even better introduction. The standard of the remaining papers is rather higher than in the usual journals, and several contain useful introductions and bibliographies for their application areas. Stallings' paper on Chinese ideograms is particularly good in this respect.

In pattern recognition one can either start with a technique and see how it works in a given application, or one can start with an application and devise suitable techniques. The present book mainly follows the first of these strategies, whereas the papers in *Digital Picture Analysis*, Edited by A. Rosenfeld (Springer, 1976) mainly follow the second strategy and turn out *not* to be straightforwardly syntactic in the pure linguistic sense. The syntactic approach is just one among many; and it has perhaps met its greatest commercial success in reading machines such as the IBM 1287 which are not mentioned in the book under review.

This book is not about established commercial applications but instead reports research explorations of conceivable applications for syntactic techniques, and does not give much idea of the relative efficacy of syntactic compared with other techniques. Nevertheless, it is as good a collection of papers as we are likely to get in 1977 on this topic.

J. R. Ullmann

J. R. Ullmann is Professor of Computing Science in the Department of Applied Mathematics and Computing Science, University of Sheffield, UK.

BOOKS

ON PURE
AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £5.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Circle No. 17 on Reader Enquiry Card.

The medium for short reports on all aspects of microbiology and microbial chemistry

FEMS Microbiology Letters

Chief editor: D.W. Tempest, Amsterdam, The Netherlands

1977 - Volumes 1 and 2 in
12 issues US \$ 81.75/Dfl.
200.00

Published on behalf of the
Federation of European
Microbiological Societies by

For a specimen copy write to:

ELSEVIER/NORTH HOLLAND
BIOMEDICAL PRESS

P.O. Box 211, Amsterdam,
The Netherlands

The Dutch guilder price is definitive. US \$ prices are subject to exchange rate fluctuations.

Circle No. 18 on Reader Enquiry Card.

Foundations of population genetics

Foundations of Mathematical Genetics. By A. W. F. Edwards. Pp. viii+119. (Cambridge University: Cambridge, London and New York, 1977.) £5.80.

JUDGING from the title, you might expect this book to be a history of population genetics. It is a fine book; but it is not a history, certainly not in the conventional sense. The book discusses the foundations of population genetics, and to A. W. F. Edwards that mainly means the single locus, infinite population, discrete generation, random mating, constant fitness model. There is some justification for focusing on this model and closely related ones, for, as Edwards points out, "... the elementary mathematical theory [of population genetics] has reached a sufficient maturity to be presented ... as a piece of mathematical standing in its own right" (page vii). The approach is purely mathematical, therefore, and the book will be useful to mathematicians and statisticians who wish to have a concise introduction to the essential terminology and concepts of population genetics. It will also be useful to population geneticists, evolutionary biologists, or ecologist who want a thorough review of some of the fundamental models of population genetics.

In elaborating in detail the implications of a few fundamental models, Edwards goes somewhat counter to what has been and remains an historical trend in population genetics, a trend toward generality at the expense of precision. How this trend became established is not completely clear, but Edwards attributes it to the genius of the founders: "One of the inevitable corollaries of the fact that the structure of mathematical genetics was created by three men of exceptional calibre, R. A. Fisher, J. B. S. Haldane and S. Wright, has been that the ground floor of the edifice is scantily furnished. They ascended rapidly to the upper storeys, not always by the same staircase" (page vii).

On the other hand, the quest for generality has characterised not only population genetics but also much of theoretical evolutionary biology. Emphasis has been on the search for principles that apply at all times and to all situations; special cases, or principles with a restricted range of validity, are anathema. What we have learned in the past fifty years is that there are precious few general principles, and these few are so abstract as to be not especially useful.

Edwards has in any event countered the trend towards a superficial analysis of a multitude of models. This seems thoroughly appropriate for a book whose purpose is to present elementary population genetics as a branch of mathematics. Whether the approach is equally valid for those whose interest is in actual, evolving populations is another matter.

In focusing on a few specific models, Edwards has discovered that, as the creators of population genetics ascended to the upper storeys, they left a number of important theoretical gaps below, in the analysis of the simple, fundamental models of population genetics. In a number of instances Edwards has plugged the gaps himself, so the book contains much that is new.

The book focuses on the discrete-generation models of selection in diallelic, triallelic, and multiple allelic autosomal loci as well as sex-linked loci; brief sections are also devoted to models with differential viabilities in

the sexes, selection in heterogeneous environments, and selection with two linked, diallelic autosomal loci. Throughout the book, emphasis is on finding conditions for the existence of polymorphic equilibria and determining when the gene frequency will actually converge to the equilibrium. Considerable attention is also given to the behaviour of the mean fitness function. One attractive feature about the book is a consistent and clever use of triangular coordinates, following a century-old treatise on the subject by Reverend N. M. Ferrers, one of Edwards' predecessors as Fellow and Tutor of Gonville and Caius College.

All in all, *Foundations of Population Genetics* is a thorough, scholarly treatise, and a useful addition to any professional's library.

Daniel L. Hartl

Daniel L. Hartl is Professor of Biology at Purdue University, West Lafayette, Indiana.

Molluscan atlas

Atlas of the Non-Marine Mollusca of the British Isles. Edited by M. P. Kerney. Pp. v+202. (Conchological Society of Great Britain and Ireland: Luton, UK, 1976.) £3.

It is ironical that a major contribution to the study of land and freshwater molluscs in the British Isles should have been published in a year when a catastrophic drought produced a major perturbation on many molluscan populations. The production of this atlas is therefore apposite in providing basis for monitoring ensuing changes. Moreover, it commemorates a century of cooperative endeavour in recording the distribution of molluscs by a succession of dedicated enthusiasts.

This volume deserves recognition by a much wider audience than malacologists, for it summarises in graphical form information collected during the most detailed survey undertaken in this country on any invertebrate group of comparable size. Indeed, it represents a milestone in the development of mapping schemes, and should provide a catalyst to other small organisations undertaking similar projects.

The distribution of 192 taxa is presented on the now conventional 10-km grid maps used by the Biological Records Centre and the information is categorised on the basis of recorded living before or after 1960.

A further refinement is included: the records of the relevant fossils are plotted for those species where there is evidence of changes in the distribution since the last Postglacial period. A promised set of transparent overlays will provide information on a range of environmental parameters and thus enhance the interpretation of the maps; these are eagerly awaited.

Results have already accrued from this survey and these are reflected in the recording of several species new to the British Isles and in the re-assessment of the distribution or taxonomic status of others. Nevertheless, the enduring value of this type of work is the enormous impetus and stimulus it should provide for a wide range of research programmes and other activities.

Many biologists and naturalists will regret the adoption of a number of unfamiliar innovations in nomenclature even though they will applaud the desire to establish uniformity of treatment throughout Europe. This laudable objective would have been more palatable by the addition, to each map, of references to more widely known synonyms. An indication of the habitat range and extra-territorial distribution of each species would have further facilitated an understanding of the recorded patterns. Such limitations, however, should not detract from the value of this atlas.

J. F. Peake

J. F. Peake is Head of the Mollusca Section and Deputy Keeper of Zoology at the British Museum (Natural History).

obituary

L. F. Cooling

DR L. F. COOLING died on Tuesday, 15 February after a short illness. He was 73. His death marks the close of a significant era in the development of engineering science in Britain. Born in Birmingham, he graduated there in physics in 1925 and later gained an MSc for studies of the thermo-magnetic properties of meteorites. In 1927 he joined the Building Research Station at Garston and he remained there until his retirement as an assistant director 41 years later in 1968.

His early work on permeability and evaporation properties of porous building materials formed a good basis for his future research into the engineering properties of the ground. In 1933 he was made head of a soil physics section formed to carry on Prof C. F. Jenkin's work on earth pressure and to study road foundation problems for the Road Research Board. His laboratory in the Stable Block at Garston was the pioneer soil mechanics laboratory in Britain.

Cooling was the only British participant at the first International Soil Mechanics Conference in Harvard in 1936 where he acquired confidence in

his subject. By 1937, when he had been joined by Prof A. W. Skempton, FRS, and Dr H. Q. Golder, he had aroused considerable interest among civil engineers in the practical value of soil mechanics by his investigations of several landslips involving railway cuttings and retaining walls. In particular his diagnosis of the Chingford Reservoir embankment failure, which brought Karl Terzaghi to England for the first time, provided the impetus for the recognition of soil mechanics as an engineering discipline throughout Britain and engineers from many organisations came to the Building Research Station to be trained.

Cooling was practically minded but he never pretended to be an engineer. His lucid understanding of physics enabled him to convince engineers of his reasoning and they had absolute confidence in him. He was unpretentious and always talked sense.

During the war years the emphasis on the investigation and analysis of foundation and earthwork failures grew. The field studies pointed to weakness in the understanding of the in-situ mechanical behaviour of the ground and Cooling initiated a programme of intensive field investigations in which

detailed measurements were made of the performance of structures such as earth dams, tunnels and building foundations.

Cooling's contributions to engineering science were recognised in 1952 by his University with the award of a DSc, this time in engineering, and by the Institution of Civil Engineers who invited him to their membership. In later life Dr Cooling played many leading roles in the committees, societies and journals concerned with the national and international organisation of soil mechanics. He gave the second Rankine Lecture "Field measurements in Soil Mechanics" to the British Geotechnical Society who also recognised his interests in young people by creating the Cooling Prize, to be awarded annually to the best presentation from a young engineer—a function he always looked forward to in his retirement.

Cooling was a keen sportsman. At University he played for the England Amateur football team. He was a Midland Counties Champion runner and an enthusiastic player of shove ha-penny and golf.

He leaves a wife and a daughter.

W. H. Ward

E. Cunningham

EBENEZER CUNNINGHAM who died in Corbridge, Northumberland, on February 12 at the age of 95 was a pioneer in introducing the work of Einstein in this country. His father was a cabinet maker; G. D. Cunningham, organist and conductor, was his elder brother. From Owen's School, Islington, he won a mathematical scholarship at St John's College, Cambridge, was Senior Wrangler in 1902 and a Smith's Prize-man in 1904, when he was elected to a Fellowship. After holding lectureships at Liverpool University and University College, London, he returned to St John's in 1911 and until 1976 his home was in Cambridge, where he played an important part both in the Faculty of Mathematics and in his college. His clear and stimulating lectures on branches of applied mathematics are remembered with pleasure and a pupil says of his approach to an unfamiliar

problem, "you were seeing the Senior Wrangler in action."

His first few papers were on pure mathematics but by 1907 he was writing on electromagnetism; in 1909–12 he and H. Bateman, for a time a colleague at Liverpool, contributed papers to the *Proceedings of the London Mathematical Society* on the four-dimensional transformations of the electro-dynamical equations. In 1910 he also made a correction to the Stokes formula for the velocity of steady fall of particles in a fluid.

For the next decade his main interest was the study and exposition of Einstein's theories. *The Principle of Relativity* (C.U.P. 1914) gave the first account in English of the Special Theory of Relativity. In 1915 he published *Relativity and the Electron Theory* (Longmans, Green & Co.) and in the second edition (1921) he included an introduction to the General Theory; "Gravitation" was added to the title.

His books emphasise comparison with theory at every stage; in most other accounts it is difficult to see how much of the theory is directly required by experimental evidence and how much, apparently, by pure thought. In 1919, very soon after the announcement on November 6 of the results of the Solar Eclipse Expeditions, he wrote three articles in *Nature* (Vol. 104) on "Einstein's Relativity Theory of Gravitation." In 1921 *Nature* for February 17 was devoted to articles on Relativity and his contribution follows that of Einstein.

He and his wife, whom he married in 1908, were active members of Emmanuel Congregational Church and they made many friends in all walks of life. He bore with courage her loss in 1969 and his increasing blindness. In this he was helped by his strong religious belief and his lifelong love of music.

Harold Jeffreys

G. M. Frank

ACADEMICIAN GLEB MIKHAILOVICH FRANK, the eminent Soviet biophysicist died on October 10, 1976.

Born in 1904, Frank graduated from the University of Simferopol in 1925. From 1929 to 1943, he worked at the Leningrad Physico-Technical Institute and then at the All-Union Institute of Experimental Medicine. From 1943–52 he carried out research in various laboratories of the Academy of Sciences of the USSR. During this time, he became increasingly associated with the Institute of Biophysics of the Academy of Sciences, becoming its Deputy Director in 1952 and Director in 1957. He was elected to the Academy of Sciences of the USSR in 1966.

Frank's research dealt mainly with the effect of ultraviolet light and ionising irradiation on animal organisms, and also the biophysical causes of nervous stimulation and muscular contractions. He made numerous innovations in photobiology, radiobiology and the biophysics of elementary life processes. He demonstrated the difference

in the biological effects of short-wave and long-wave ultraviolet radiation, showing that, in the first case photodenaturation predominates, and in the second photolysis. His work on ultraviolet radiation found numerous applications in Soviet medical practice, including ultraviolet anaesthesia and the treatment of traumas of the peripheral nervous system.

Frank played a leading part in the development of *in vivo* studies of early physico-chemical and functional changes in tissues exposed to ionising radiation. He identified a number of phenomena occurring during irradiation, and developing in the latent post-irradiation period (depression, variations in the oxygen utilisation pattern, changes in the erythrocytes, and in the electrical parameters of the tissues). He also developed the concept of negative feedback in cellular processes.

In 1927, Frank published a paper showing longitudinal sections of muscle fixed at rest and during isometric contraction at various initial lengths. He obtained the striking result that in con-

tracting muscle, the width of the A bands was independent of muscle length. This was a foreshadowing of the results which in 1953–54 contributed to the establishment of the sliding-filament theory of muscle contraction. His continued interest in this area was seen when he attended the Discussion Meeting on Muscular Contraction at the Royal Society in November 1963.

His major works include *Approaches to the in vivo investigation of the physico-chemical processes of nervous activity* (1954), *Early reactions of the organism to irradiation in dependence on local perturbations* (1955), and *Physico-chemical and structural principles of biological processes* (1960). He was co-editor of the radio-biology section of the second edition of the Soviet *Large Medical Encyclopaedia*.

For his services to Soviet Science and Medicine, Frank was twice awarded the Order of Lenin (the highest civil award) and four times the Order of the Red Banner of Labour. His research twice received a Stalin (now State) Prize. *Vera Rich*

Sheina Marshall

DR SHEINA M. MARSHALL, OBE, FRS, died at Millport on 7 April 1977.

After holding a Carnegie Scholarship at Glasgow University, during which she showed her research abilities in a study of the behaviour and structure of *Hydra*, she joined the staff of the Scottish Marine Biological Association's laboratory at Millport in 1922. Here she started immediately in the field of research in which she was to be engaged for the rest of her life with the most detailed study yet made on the feeding of the copepod *Calanus* and a description of a new species of dinoflagellate.

She then began a remarkable collaboration with A. P. Orr, chemist on the Millport staff, through which they became known internationally as pioneers in the study of marine production. Starting with experiments on the photosynthesis of diatom cultures in bottles submerged in the sea, they proceeded to their classic investigation of the seasonal changes in nutrients and phytoplankton in Loch Striven. In this work there can be seen the seeds from which much of the modern research on productivity has developed.

They then turned their attention to the biology of *Calanus* and other smaller copepods which form a major constituent of the zooplankton responsible for secondary production. In part

of the work on *Calanus* they were joined by A. G. Nicholls. A full study was made at sea of the general biology of these copepods, including their vertical distribution, successive generations throughout the year, their methods of feeding and chemical composition. This was later supplemented by experimental observation in the laboratory on respiration, food requirements, and excretion both in the adult and all young stages. By the use of radioactive phosphorus they showed its very rapid transfer to the ovaries and developing eggs and embryos, thus confirming their theory that abundant food is necessary for egg laying resulting in an adaptation whereby the hatching of the nauplii coincides with diatom outbursts.

After the death of Orr in 1962 Sheina Marshall started further collaboration with E. D. S. Corner, biochemist at the Plymouth laboratory of the Marine Biological Association. Thus her remarkable expertise and knowledge of copepod biology has been made available to a new generation of workers in the field of marine production. Her last investigation was concerned with the overwintering generation of *Calanus* in which it was shown that a partially carnivorous diet is probably necessary for survival.

In 1928 Marshall and Orr went with the Great Barrier Reef Expedition to Australia. There they undertook a similar seasonal investigation to that

made in Loch Striven on the nutrient and phytoplankton cycles in the reef area. This was the first to be done on this scale in tropical waters. Sheina, always indefatigable, made observations also on the occurrence of symbiotic algae in coral planulae, and with Orr investigated the ability of certain corals to rid themselves of silt.

On their return to Millport they made a short investigation, with A. G. Nicholls, on the growth of the early stages of the herring in the Clyde Sea Area, which is probably the most complete observation made on a single brood.

During the war she was engaged on two projects in the applied field, namely the fertilisation of sea lochs and the production of agar from the seaweed *Gigartina*.

Although not a systematist she had a special interest in tintinnids on which she wrote a Barrier Reef report and prepared plankton identification sheets for the International Council for the Exploration of the Sea.

Her work with Orr was brought together in their book *The Biology of a Copepod*, and she amplified and prepared for press Orr's Buckland lectures given in 1957 to form the book *The Fertile Sea*.

Much travelled, her loss will be felt by many friends all over the world who got to know her sterling qualities.

F. S. Russell

what's new – microscopes

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues. The issue of 18 August will be on Chromatographs and ancillary equipment and that of 20 October on Spectrometers and ancillary equipment.

The purpose of a special feature will not be to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom.

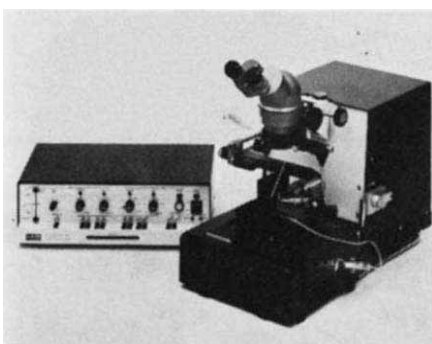
This feature on Microscopes and ancillary equipment covers optical and electron microscopes, stereomicroscopes, wide-field and measuring microscopes, teaching microscopes, stages, ultratomes and cryomicrotomes, fluorescence and metallurgical microscopes and other related items.

Available from **Philips** is a high-performance scanning electron microscope PSEM 500 interfaced with a fully-focusing wavelength dispersive X-ray spectrometer, the PSEM 500X, not only permits analysis of X-rays from $Z=5$ to $Z=92$ but is the first such system to use the vertical spectrometer orientation—a configuration which offers the highest mechanical reproducibility—without the need for an optical microscope. Instead, a new standard in specimen height accuracy and reproducibility is achieved via the unique, fully-eucentric goniometer coupled with its 4.6 mm fine Z-adjustment (in 0.2 μm steps) and the large (210 mm) Rowland circle of the spectrometer. An extremely high-vacuum system (10^{-6} torr, inclusive of the spectrometer assembly) vastly improves practical light-element detection. Thus, absorption of 'soft' X-rays from light elements, due to specimen contamination, is seldom encountered. The same vacuum system, without modification, allows operation with optional higher-brightness sources—a particularly valuable facility for excitation of X-rays from light elements. The standard 50 kV electron optics of the PSEM 500 is ideal, when using an energy dispersive X-ray detection system, for unambiguous excitation of K-line X-rays from heavier elements.

The larger excited volume of X-rays, when using 50 kV electrons, can also facilitate easier trace-element detection. The energy dispersive and wavelength dispersive spectrometers can be in operation at the same time, thus giving an optimum choice of the characteristics of each type of spectrometer.

Circle No. 45 on Reader Enquiry Card

LKB Instruments Ltd present an advanced ultramicrotome and a new 'whole animal' microtome/cryomicrotome. The Ultratome IV is the latest, most advanced ultramicrotome developed and manufactured by LKB-Produkter in Sweden. It will prepare large-area thick epoxy sections for scanning transmission or high voltage electron microscopy; and serial ultrathin sections for transmission electron microscopy. A system of push buttons allows knife alignment and approach to the specimen to be conducted entirely from the control panel. The gravity-powered cutting stroke ensures constant and reproducible cutting speeds regardless of section size or



LKB Ultratome IV

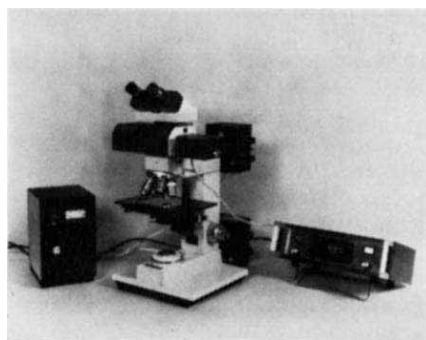
thickness, and this, in conjunction with the magnetic coarse feed, will prepare large, even sections for optical microscopy. Likewise, when used with fine thermal feed, the Ultratome will produce grid-sized sections to any thickness required between 0 and 200 nm. Other advances include specimen trimming and a new viewing system for selected area trimming; the orientation head offers precision of adjustment and provides wide specimen orientation possibilities. Ten glass knives can be evaluated at one time for edge quality and angle. There are new collection facilities for both thick and ultra-thin

sections. The PMV Cryomicrotome (made for LKB by Palmstiernas Mekaniska Verkstad, Stockholm) is a heavy-duty microtome in a cryocabinet. Specimen size need no longer be a limitation with the PMV since material of up to 45 cm $\times$ 15 cm can be sectioned. Therefore experiments can be carried out using larger animals, a feature which could provide obvious advantages in the testing of new pharmaceutical products for eventual human use. Simple operation giving reliable and reproducible results identifies the PMV approach.

Circle No. 46 on Reader Enquiry Card

Vickers Instruments, a division of Vickers Limited, manufacture a comprehensive range of optical microscopes supplemented by selected instruments from a sister Company, Nachet of Franche, and from Union Optical of Tokyo. The Company also acts as sole UK agent for Metallographic and Mineralogical specimen preparation equipment by Struers of Copenhagen. Many recent innovations include an inexpensive closed-circuit television attachment suitable for routine industrial applications as well as the more traditional teaching role; a direct-reading micro-measurement system based on the image-shearing principle and capable of sub-micrometre accuracy even in inexperienced hands; the Nachet NS400 Research microscope featuring an optical system which allows all techniques to be undertaken using a single set of objectives, and the Union Versamet, an inverted Metallographic instrument allowing instant selection of bright field, polarisation, dark ground or Nomarski interference contrast. The most significant development has, however, been the introduction of a completely new series of instruments, collectively titled M17, for research and routine applications in both Materials and Life Sciences. The M17 series is designed to accept many interchangeable accessories but the availability of several basic microscope stands allows the user to select precisely those facilities which are required. The basic range already includes purpose-built models for incident light, transmitted light and dual illumination (one offering an extra-wide 25 mm field of view) while a totally

new incident fluorescence illuminator combines simplicity of operation with true research capability. A routine fluorescence microscope is also available and instruments for analytical work in polarised light are to be released soon. The flat-field objectives, both achromats and apochromats, are completely new designs, giving improved colour corrections over the widest 25 mm field of view. A comprehensive range of fully interchangeable camera equipment, including electronic exposure units, may be fitted to any model from the M17 series; particular features of the system being the retention of full binocular vision during exposure, the absence of the usual auxiliary focusing adjustment and the facility to rotate both framing graticule and camera body to capture oblique features of the specimen.



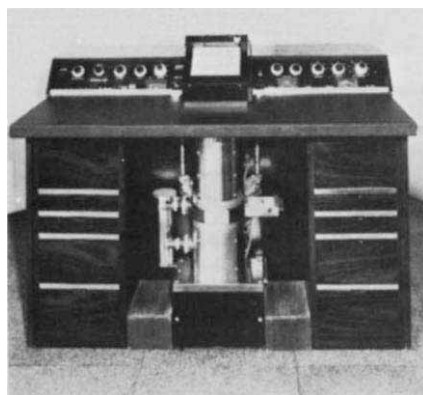
Vickers M17

Circle No. 47 on Reader Enquiry Card

Gillett & Silbert produce a broad range of microscopes from the student to the research level with the emphasis on those used routinely in the laboratory; the range includes photographic systems and a £100,000 consignment was recently delivered to Korea.

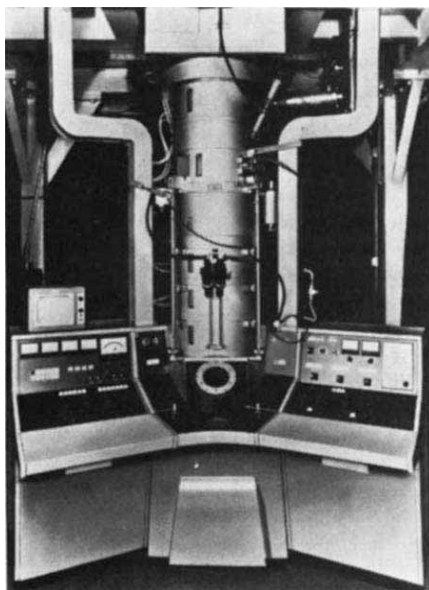
Circle No. 48 on Reader Enquiry Card

Kratos Ltd, AEI Scientific Instruments (formerly AEI Scientific Instruments) are long-time manufacturers of electron microscopes, two of which are featured here. The Corinth 500 is a high resolution electron microscope with 5 Å resolution imaging, multiple specimen facilities and multiple accelerating voltages of 20, 40, 60 and 80 kV. A double condenser lens system reduces specimen damage; there are excellent viewing facilities— $\times 240,000$ top magnification and excellent recording facilities—70 mm roll film camera is standard equipment. The Corinth has very high stability supplies— 5×10^{-6} per min and a foolproof fully automatic vacuum system. Also X-ray leakage rate is very low—less than 0.1 mR h^{-1} . Additional optional extras include viewing hood for daylight working, scanning attachment for reflection scanning, electron imaging of larger specimens,



AEI Corinth 500

solid state detector attachment for simultaneous transmission imaging and X-ray analysis of selected areas. Because of the high penetration of the electron beam, much thicker specimens can be investigated in a high-voltage electron microscope than in a 'conventional' instrument operating with an accelerating voltage of 100 kV. For this reason in particular, metallurgists and material scientists in the 1960s were the first to take advantage of the high-voltage technique. The thicker specimens which could be used were more representative of the bulk material from which they were prepared and they consequently revealed information about the material which could not be obtained by any other means. The high-voltage microscope also provides the biologist with facilities to exploit the exciting technique of stereoscopic micrographs. The thick sections of biological material make ideal subjects for viewing and photographing from two different angles, and, for the first time, it has been possible to record effective three-dimensional images which provide quite remarkable information and a new



AEI EM7 Mk II

understanding of structure. One of the most recent applications of the high-voltage microscope is in geological research. Using specimens prepared by the ion beam technique, only a small part of the thinned section can be investigated with conventional electron microscopes. But, with a million-volt instrument, information can be obtained from a far greater area of the section which is more representative of the bulk material and the advantages of the technique hold promise for many new far-reaching discoveries in this field. The EM7 Mk II high-voltage electron microscope has a 5 Å resolution, a wide operating voltage range—100–1250 kV, can be used for critical voltage experiments and radiation damage experiments. It has wide magnification range— $\times 63$ to $\times 1,250,000$ and is suitable for grid searching and high resolution applications. Rotation free imaging— $\times 4000$ to $\times 1,000,000$ enables an area to be viewed with zoom magnification without the image rotating. Operation is very easy, all controls are conveniently placed, specimen chamber is within easy reach of operator. There are excellent viewing and recording facilities and accessories available are solid-state elemental analyser, closed-circuit TV system, image intensifier, radiation monitors, plate desiccator, liquid helium anticontaminator, high resolution diffraction stage, vacuum coating unit.

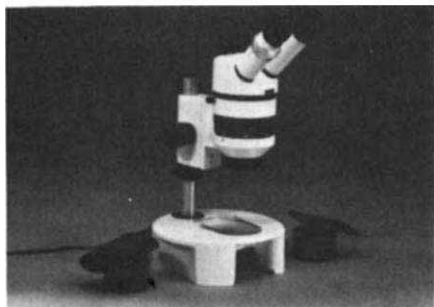
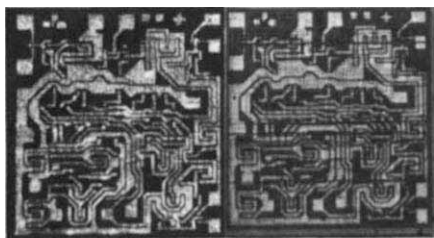
Circle No. 49 on Reader Enquiry Card

Wild Heerbrugg Ltd, Switzerland, present a photomicroscope, the Wild M400. This system opens up even to the non-specialist the hitherto rather difficult photographic magnification range from 1:1 to 60:1. Binocular



Wild M400

observation, zoom optics 1:5 and completely automatic exposure and film transport allow the observer to concentrate entirely on the specimen and format limits. Owing to its modular construction with different stands and other accessories for incident light,



Wild M5A

transmitted light, bright field and dark field, polarisation etc, the Wild M400 can meet all the demands of present-day macroscopy. The new Wild M5A Stereomicroscope has several new design features, particularly the availability of a unique new coaxial incident illuminator which directs the light of a 20 W halogen illuminator along the two optical trains, with the result that the image is free from the glare which is often so troublesome in the examination of highly-reflecting metallurgical specimens or of multilayer thin-film materials such as integrated electronics circuits. Additional objectives and interchangeable eyepieces extend the magnification range to $\times 250$. A metal hood, which unites with the baseplate, protects the instrument from dirt and from transport damage. The modular design enables a variety of observation techniques and supplementary sets of accessories (for example for photomicrography and television microscopy) to be applied.

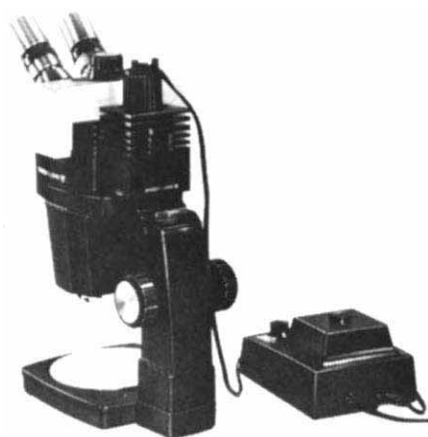
Circle No. 50 on Reader Enquiry Card

Available from **Stanton Redcroft** is a hot stage microscope unit, the HSM-5, a unit which enables the user to observe materials under controlled conditions over the temperature range ambient to 1000°C . The unit may be used with any large working distance microscope especially with a stereoscopic microscope. 35 mm photomicrography and time lapse and high speed ciné photography are readily possible. The sample temperature and the intensity of the light reflected from the sample can be accurately followed. The sample is held in a standard DTA sample pan. Specification: water-cooled micro-furnace with temperature range ambient to 1000°C . Rapid temperature response and cooling from

1000°C to below 100°C in less than 5 min; wide range of switch selected linear heating (and cooling) rates from 1 to 100°C per min. Rapid establishment of isotherms to within $\pm 1^\circ\text{C}$. Full atmosphere control, small swept volume for ready coupling to a gas analysis system. Accurate sample temperature measurement using plate type Pt v Pt-13% Rh thermocouple. No special sample preparation necessary. Quartz window for viewing. Can be rotated to remove condensed volatiles from the field of view. The unit has been used to study a range of reactions including the fusion and decomposition of organic and inorganic materials, oxidation of metal powders, the behaviour on ignition of pyrotechnic mixtures.

Circle No. 51 on Reader Enquiry Card

Bausch & Lomb UK Ltd market a wide variety of microscopes, stereomicroscopes and stereozoom microscopes for use in education, industry, electronics, R&D, medicine and the Laboratory. The **ESM 25/100** Microscope is ideal for elementary science teaching, as well as a highly practical instrument for field observations. It combines two magnifications in one instrument, the $\times 25$ position for low power scanning and for macro specimens up to 10 mm thick, the $\times 100$ position for higher power detailed study. Model **SSM** Student Stereomicroscopes ($\times 15$) and Model **ST2** Science Teaching Zoomscopes ($\times 50$ to $\times 200$) are both low-priced durable student microscopes. The **Academic Stereozoom** series of microscopes are also designed for science teaching, with zoom optics, 'student proof' construction, king-size stage and 'dial a light' facility—a three-way illumination system giving a choice of reflected light, transmitted light or a combination. The **Stereozoom Series (5 and 7)** with exclusive **Power Pod** gives real

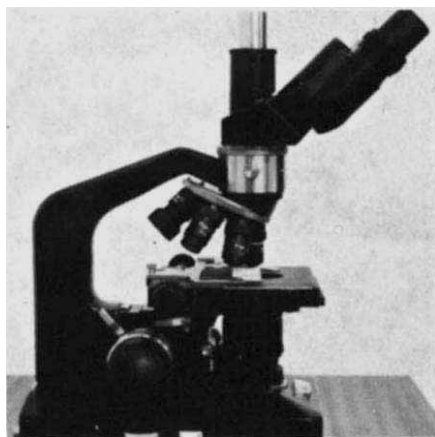


Bausch & Lomb Stereozoom

3-dimensional images by means of the Greenough design which features two complete converging optical systems that have a uniform optical path from eyepiece to objective. Both systems are aligned to focus at a single point assuring true 3-dimensional imagery, as each system fully utilises its own lenses. Zoom optics are standard and the sealed **Power Pod** prevents dust from entering the optical system, also stops image jump and image blackout. A flat-field optical system is integral and flexibility of attachment options covers a variety of **Power Pods**, eyepiece magnifications, supplementary lenses, stands, illuminators, polarising and photographic accessories.

Circle No. 52 on Reader Enquiry Card

The **Tiyoda Super Widefield MT-B** Microscope, available from **Hall Bros (Optical) Ltd** is a new concept in microscopy. This microscope is unique in design and construction, the designers having consulted professionals from all branches of medicine and science. Super Widefield eyepieces allow fatigue-free viewing of a field 90% larger than that seen with conventional widefield

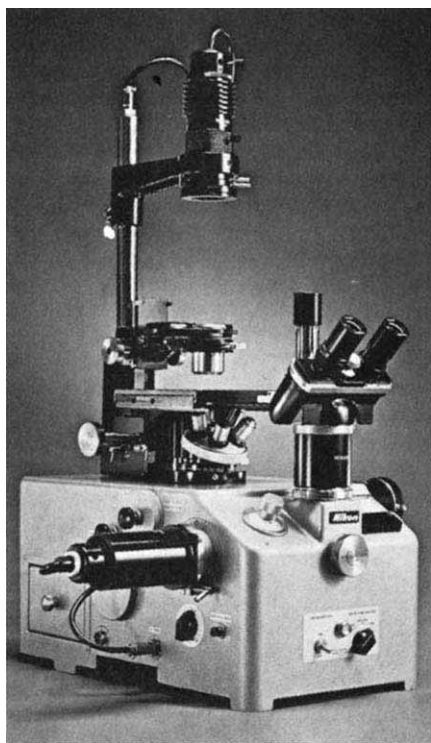


Hall Bros Tiyoda MT-B

eyepieces and a new patented 11 element Apochromatic condenser complements the Plan Apochromatic objectives $\times 2$, $\times 4$, $\times 10$, $\times 20$, $\times 40$ and $\times 60$. With these there is a $\times 1$ Plan Achromat which is perfectly par-focal with the other objectives. All controls are grouped together in one zone, letting the operator control the focus, aperture, stage movement and condenser adjustments with ease. For the first time super widefield photography is possible. A $\times 0.66$ lens is built into the phototube, and a low power photo-eyepiece combines to cover a rectangular format extending right to the edge of the visual field, e.g. with the Plan Achromat $\times 1$ objective an area of 14×22 mm can be recorded on 35 mm film.

Circle No. 53 on Reader Enquiry Card

Nikon manufacture a wide range of microscopes and ancillary equipment; research microscopes of various types—the Apophot, the Multiphot and the Biophot, conventional optical microscopes (the L-Ke, S-Ke and S-kT ranges), polarising (Model POH-3) and fluorescence (Model FL/FT) microscopes, stereoscopic (Model SM), metallurgical (Models ME, MSE, S-Epi, hole and surface-finish microscopes), inverted (Models M, MS and MTD), photo-ciné micrographic equipment and a wide range of interchangeable accessories. The Apophot has a sturdy console construction, a trinocular eyepiece tube, built-in magnification



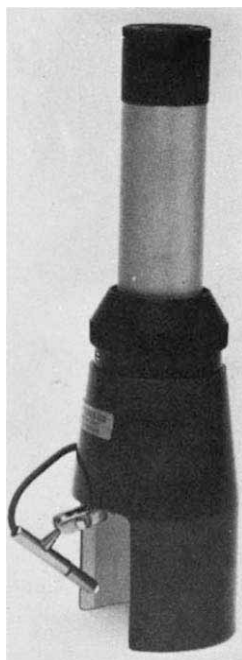
Nikon Inverted Microscope

changer, interchangeable nosepiece system, a variety of stages, interchangeable condenser system, zoom Koehler illumination and various light sources available, Phase contrast (PC), interference-phase (IP), differential interference (DI) and polarising (P) attachments, also photomicrographic (PM) and ciné facilities and optional projection screen ($\times 6$ to $\times 3,000$). The Multiphot is capable of large-format photomacro- and photomicrophotography. The Biophot can be used for photomicrography, has a trinocular nosepiece tube, interchangeable arms, six-objective revolving nosepiece, filter facility, large stage and swing-out condenser, Koehler illumination, interchangeable eyepiece tubes and stages, P, IP, DI and PM facilities. The M, MS and MTD range of inverted microscopes, are particularly useful for tissue culture research. Interchangeable eyepiece tubes, PC, IP, DI and P facilities, also cine PM attachments, projection

screens and a specimen incubator. (Model M magn. $\times 6$ to $\times 2,000$). Model MS has a diascope illuminator and can be supplied in a biological or metallurgical form ($\times 30$ to $\times 2,250$; for PM, $\times 20$ to $\times 1,500$). Model MTD is specifically for tissue culture research ($\times 100$ to $\times 200$). Models ME, MSE, S-Epi (metallurgical) have magnifications in the range $\times 25$ to $\times 2,700$. The SM range of stereoscopic microscopes include the SM-5 ($\times 10$ to $\times 60$), the SMZ-2 stereoscopic zoom microscope ($\times 4$ to $\times 120$), the SMZ-6 ($\times 45$ to $\times 120$), various bases, pillars, stages, stands and PM facilities. The POH-3 polarising microscope has an extra long range of stage elevation, interchangeable single and multiple nosepieces, built-in transformer and Koehler illuminator, interchangeable eyepiece tubes, graduated, circular rotatable stage and epi-illuminator. Models FL/FT fluorescence microscopes have all types of filters, PC and PM facilities. **Circle No. 54 on Reader Enquiry Card**

Graticules Ltd have a comprehensive range of eyepiece and stage micrometers, counting and sizing graticules and the England Finder. Recently developed products include measuring instruments incorporating graticules, pocket measuring magnifiers and portable measuring microscopes. The Co-ordinate Measuring Microscope is a travelling microscope allowing measurements to be made over an area of $250\text{ mm} \times 250\text{ mm}$. Accessories include a rotary work table, light box, electronic digital readout, projector or 45° viewing heads and a choice of objectives, eyepieces and graticules.

The Portable Measuring Microscopes (Series 800) are versatile hand held microscopes calibrated to read down to

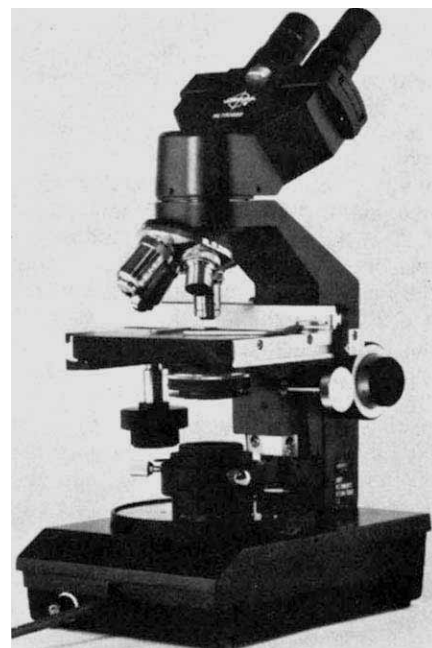


Graticules Series 800

$5\text{ }\mu\text{m}$. Accessories include miniature light fittings, spray droplet analysing gratitudes and plated through hole optics sets. The series 800 are designed for use on the work bench, in the laboratory, drawing office and so on, ideal anywhere where a full size microscope would be inconvenient to use and difficult to set up.

Circle No. 55 on Reader Enquiry Card

Pyser Ltd. for both teaching and research applications, the Swift M1000E series microscopes have a newly-designed base and a low-cost phase contrast unit. The base incorporates pre-centred 6 V 20 W built-in halogen illumination, the brightness of which can be adjusted by means of variable light control. The semi-Koehler illumination system is fitted with a centerable field iris; an alternative mains base with a built-in 240 V 30 W lamp can be supplied. Monocular, binocular,



Pyser Swift M1000 E

trinocular and tutor-tube versions are available; also a wide choice of stages to meet varying budget requirements. The plain stage is drilled to take stage clips or an attachable mechanical stage with controls on the top; a built-on mechanical stage has coaxial controls at the side while another built-on mechanical type has low-position vertical drive coaxial knobs. Coarse and fine focusing operates on the stage, which moves on precision ball bearings. The coarse adjustment is fitted with a patented clutch. These instruments can be used for dark and bright ground microscopy as well as phase contrast. In addition to the normal ranges of achromats and plan achromats, new objectives of $\times 40$ and $\times 100$ have been introduced for both bright field and phase contrast in the semi-plan form. **Circle No. 56 on Reader Enquiry Card**